(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 January 2004 (15.01.2004)

PCT

(10) International Publication Number
WO 2004/005513 A2

(51) International Patent Classification[7]: C12N 15/11, A61K 38/00

(21) International Application Number: PCT/IB2003/003052

(22) International Filing Date: 12 June 2003 (12.06.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/189,818 3 July 2002 (03.07.2002) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2004/005513 A2

(54) Title: METHODS FOR SPECIFICALLY INHIBITING HISTONE DEACETYLASE-7 AND 8

(57) Abstract: This invention relates to the inhibition of histone deacetylase (HDAC) expression and enzymatic activity. The invention provides methods and reagents for inhibiting HDAC-7 and HDAC-8 by inhibiting expression at the nucleic acid level or inhibiting enzymatic activity at the protein level.

METHODS FOR SPECIFICALLY INHIBITING HISTONE DEACETYLASE-7 AND 8

(Case No. MET-024PC)

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the fields of molecular biology and medicine. More specifically, the invention relates to the fields of gene expression and oncology.

Summary of the Related Art

Chromatin is the complex of proteins and DNA in the nucleus of eukaryotes. Chromatin proteins provide structural and functional organization to nuclear DNA. The nucleosome is the fundamental unit of structural organization of chromatin. The nucleosome principally consists of (1) the core histones, termed H2A, H2B, H3, and H4, which associate to form a protein core particle, and (2) the approximately 146 base pairs of DNA wrapped around the histone core particle. The physical interaction between the core histone particle and DNA principally occurs through the negatively charged phosphate groups of the DNA and the basic amino acid moieties of the histone proteins. (Csordas, *Biochem. J.*, **286**:23-38 (1990)) teaches that histones are subject to posttranslational acetylation of their epsilon-amino groups of *N*-terminal lysine residues, a reaction that is catalyzed by histone acetyl transferase (HAT). The posttranslational acetylation of histones has both structural and functional, *i.e.*, gene regulatory, consequences.

Acetylation neutralizes the positive charge of the epsilon-amino groups of *N*-terminal lysine residues, thereby influencing the interaction of DNA with the histone core particle of the nucleosome. Thus, histone acetylation and histone deacetylation (HDAC)

are thought to impact chromatin structure and gene regulation. For example, Taunton *et al.*, *Science,* **272**:408-411 (1996), teaches that access of transcription factors to chromatin templates is enhanced by histone hyperacetylation. Taunton *et al.* further teaches that an enrichment in underacetylated histone H4 has been found in transcriptionally silent regions of the genome.

Studies utilizing known HDAC inhibitors have established a link between acetylation and gene expression. Yoshida *et al*, *Cancer Res.* **47**:3688-3691 (1987) discloses that (R)-Trichostatin A (TSA) is a potent inducer of differentiation in murine erythroleukemia cells. Yoshida *et al.*, *J. Biol. Chem.* **265**:17174-17179 (1990) teaches that TSA is a potent inhibitor of mammalian HDAC.

Numerous studies have examined the relationship between HDAC and gene expression. Taunton *et al.*, *Science* **272**:408-411 (1996), discloses a human HDAC that is related to a yeast transcriptional regulator. Cress *et al.*, *J. Cell. Phys.* **184**:1-16 (2000), discloses that, in the context of human cancer, the role of HDAC is as a corepressor of transcription. Ng *et al.*, *TIBS* **25**: March (2000), discloses HDAC as a pervasive feature of transcriptional repressor systems. Magnaghi-Jaulin *et al.*, *Prog. Cell Cycle Res.* **4**:41-47 (2000), discloses HDAC as a transcriptional co-regulator important for cell cycle progression.

The molecular cloning of gene sequences encoding proteins with HDAC activity has established the existence of a set of discrete HDAC enzyme isoforms. Grozinger *et al.*, *Proc. Natl. Acad. Sci. USA*, **96**:4868-4873 (1999), teaches that HDACs may be divided into two classes, the first represented by yeast Rpd3-like proteins, and the second represented by yeast Hda1-like proteins. Grozinger *et al.* also teaches that the human HDAC-1, HDAC-2, and HDAC-3 proteins are members of the first class of HDACs, and discloses new proteins, named HDAC-4, HDAC-5, and HDAC-6, which are members of

the second class of HDACs. Kao *et al.*, *Gene & Development* **14**:55-66 (2000), discloses an additional member of this second class, called HDAC-7. More recently, Hu, E. *et al.* J. Bio. Chem. **275**:15254-13264 (2000) discloses the newest member of the first class of histone deacetylases, HDAC-8. It has been unclear what roles these individual HDAC enzymes play.

Known inhibitors of mammalian HDAC have been used to probe the role of HDAC in gene regulation for some time. Yoshida *et al.*, *J. Biol. Chem.* **265**:17174-17179 (1990) discloses that (R)-Trichostatin A (TSA) is a potent inhibitor of mammalian HDAC. Yoshida *et al*, *Cancer Res.* **47**:3688-3691 (1987) discloses that TSA is a potent inducer of differentiation in murine erythroleukemia cells.

Known inhibitors of histone deacetylase are all small molecules that inhibit histone deacetylase activity at the protein level. Moreover, all of the known histone deacetylase inhibitors are non-specific for a particular histone deacetylase isoform, and more or less inhibit all members of both the histone deacetylase families equally. (Grozinger, C.M., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**:4868-4873 (1999)). For example, see Marks *et al.*, *J. National Cancer Inst.* **92**:1210-1216 (2000), which reviews histone deacetylase inhibitors and their role in studying differentiation and apoptosis.

Therefore, there remains a need to develop reagents for inhibiting specific histone deacetylase isoforms. There is also a need for the development of methods for using these reagents to modulate the activity of specific histone deacetylase isoforms and to identify those isoforms involved in tumorigenesis and other proliferative diseases and disorders.

BRIEF SUMMARY OF THE INVENTION

The invention provides methods and reagents for modulating the activity of histone deacetylase (HDAC) isoforms. For example, the invention provides methods and reagents for inhibiting HCAC isoforms, particularly HDAC-7 and HDAC-8, by inhibiting expression at the nucleic acid level or enzymatic activity at the protein level. The invention provides for the specific inhibition of specific histone deacetylase isoforms involved in tumorigenesis and thus provides a treatment for cancer. The invention further provides for the specific inhibition of particular HDAC isoforms involved in cell proliferation, and thus provides a treatment for cell proliferative diseases and disorders.

The inventors have made the surprising discovery that the specific inhibition of HDAC-7 and 8 dramatically induces apoptosis and growth arrest in cancerous cells. Accordingly, in a first aspect, the invention provides agents that inhibit the activity of the HDAC-7 and HDAC-8 isoforms.

In certain preferred embodiments of the first aspect of the invention, the agent that inhibits the HDAC-7 and HDAC-8 isoforms is an oligonucleotide that inhibits expression of a nucleic acid molecule encoding the HDAC-7 and HDAC-8 isoforms. The nucleic acid molecule encoding the HDAC-7 and HDAC-8 isoforms may be genomic DNA (*e.g.*, a gene), cDNA, or RNA. In some embodiments, the oligonucleotide inhibits transcription of mRNA encoding the HDAC-7 or HDAC-8 isoforms. In other embodiments, the oligonucleotide inhibits translation of the HDAC-7 or HDAC-8 isoforms. In certain embodiments the oligonucleotide causes the degradation of the nucleic acid molecule.

In a preferred embodiment thereof, the agent of the first aspect of the invention is an antisense oligonucleotide complementary to a region of RNA that encodes a portion of HDAC-7 or HDAC-8 or to a region of double-stranded DNA that encodes a portion of HDAC-7 or HDAC-8 isoforms. In one embodiment thereof, the antisense

oligonucleotide is a chimeric oligonucleotide. In another embodiment thereof, the antisense oligonucleotide is a hybrid oligonucleotide. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 13 to about 35 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In still yet another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 15 to about 26 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 20 to about 26 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 13 to about 35 nucleotides and which comprises the nucleotide sequence of SEQ ID NO: 2. In still yet another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 15 to about 26 nucleotides and which comprises the nucleotide sequence of SEQ ID NO: 2. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 20 to about 26 nucleotides and which comprises the nucleotide sequence of SEQ ID NO:2. In another embodiment thereof, the antisense oligonucleotide is SEQ ID NO: 3. In another embodiment thereof, the antisense oligonucleotide has one or more phosphorothioate internucleoside linkages. In another embodiment thereof, the antisense oligonucleotide further comprises a length of 20-26 nucleotides. In still another embodiment thereof, the antisense oligonucleotide is modified such that the terminal four nucleotides at the 5' end of the oligonucleotide and the terminal four nucleotides at the 3' end of the oligonucleotide each have 2' -O- methyl groups attached to their sugar residues.

In certain preferred embodiments of the first aspect, the agent that inhibits the HDAC-7 and/or HDAC-8 isoform in a cell is a small molecule inhibitor that inhibits expression of a nucleic acid molecule encoding HDAC-7 or HDAC-8 isoform or activity of the HDAC-7 and/or HDAC-8 protein.

In a second aspect, the invention provides a method for inhibiting HDAC-7 and/or HDAC-8 activity in a cell, comprising contacting the cell with a specific inhibitor of HDAC-7 and/or HDAC-8, whereby HDAC-7 and/or HDAC-8 activity is inhibited. In an embodiment thereof, the invention provides method for inhibiting the HDAC-7 or HDAC-8 isoform in a cell, comprising contacting the cell with an antisense oligonucleotide complementary to a region of RNA that encodes a portion of HDAC-7 or HDAC-8 or to a region of double-stranded DNA that encodes a portion of HDAC-7 or HDAC-8, whereby HDAC-7 or HDAC-8 activity is inhibited. In one embodiment thereof, the cell is contacted with an HDAC-7 or HDAC-8 antisense oligonucleotide that is a chimeric oligonucleotide. In another embodiment thereof, the cell is contacted with an HDAC-7 or HDAC-8 antisense oligonucleotide that is a hybrid oligonucleotide. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 13 to about 35 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In still yet another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 15 to about 26 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 20 to about 26 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In yet another embodiment thereof, the cell is contacted with an HDAC-7 antisense oligonucleotide that has a nucleotide sequence length of from about 13 to about 35 nucleotides and which comprises the nucleotide sequence of SEQ ID NO: 3. In another embodiment thereof, the cell is contacted with an HDAC-8 antisense oligonucleotide that has a nucleotide sequence length of from about 15 to about 26 nucleotides and which comprises the nucleotide sequence of SEQ ID NO: 4. In another embodiment thereof, the cell is contacted with an HDAC-8 antisense oligonucleotide that is SEQ ID NO: 4. In another embodiment thereof, the inhibition of HDAC-7 or HDAC-8 activity leads to the inhibition of cell proliferation in the contacted cell. In another embodiment thereof, the

inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to growth retardation of the contacted cell. In another embodiment thereof, the inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to growth arrest of the contacted cell. In another embodiment thereof, the inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to programmed cell death of the contacted cell. In another embodiment thereof, the inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to necrotic cell death of the contacted cell. In certain embodiments thereof, the cell is a neoplastic cell which may be in an animal, including a human, and which may be in a neoplastic growth. In certain preferred embodiments, the method further comprises contacting the cell with an HDAC-7 and/or HDAC-8 small molecule inhibitor that interacts with and reduces the enzymatic activity of the HDAC-7 and or HDAC-8 histone deacetylase isoform. In some embodiments thereof, the histone deacetylase small molecule inhibitor is operably associated with the antisense oligonucleotide.

In a third aspect, the invention provides a method for inhibiting neoplastic cell proliferation in an animal, comprising administering to an animal having at least one neoplastic cell present in its body a therapeutically effective amount of a specific inhibitor of HDAC-7 and/or HDAC-8, whereby neoplastic cell proliferation is inhibited in the animal. In an embodiment thereof, the invention provides a method for inhibiting neoplastic cell growth in an animal, comprising administering to an animal having at least one neoplastic cell present in its body a therapeutically effective amount of the antisense oligonucleotide of the first aspect of the invention with a pharmaceutically acceptable carrier for a therapeutically effective period of time. In an embodiment thereof, the animal is administered a chimeric HDAC-7 or antisense oligonucleotide. In another embodiment thereof, the animal is administered a hybrid HDAC-7 or HDAC-8 antisense oligonucleotide. In another embodiment thereof, the antisense oligonucleotide has a

nucleotide sequence of from about 13 to about 35 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In still yet another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 15 to about 26 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 20 to about 26 nucleotides selected from the nucleotide sequence of SEQ ID NO:1. In another embodiment thereof, the animal is administered an HDAC-7 antisense oligonucleotide having a nucleotide sequence of from about 13 to about 35 nucleotides and which comprises the nucleotide sequence of SEQ ID NO: 3. In another embodiment thereof, the animal is administered an HDAC-8 antisense oligonucleotide having a nucleotide sequence of from about 15 to about 26 nucleotides and which comprises the nucleotide sequence of SEQ ID NO:3. In another embodiment thereof, the animal is administered an HDAC-8 antisense oligonucleotide that is SEQ ID NO: 4. In another embodiment thereof, the animal is a human. In another embodiment thereof, the method further comprises administering to an animal a therapeutically effective amount of an antisense oligonucleotide complementary to a region of RNA that encodes a portion of HDAC-1 or double-stranded DNA that encodes a portion of HDAC-1. In an embodiment thereof, the animal is administered a chimeric HDAC-1 antisense oligonucleotide. In another embodiment thereof, the animal is administered a hybrid HDAC-1 antisense oligonucleotide. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 13 to about 35 nucleotides selected from the nucleotide sequence of SEQ ID NO: 5. In still yet another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 15 to about 26 nucleotides selected from the nucleotide sequence of SEQ ID NO: 5. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 20 to about 26 nucleotides selected from the nucleotide sequence of SEQ ID NO: 5. In another embodiment thereof, the animal is administered an HDAC-1 antisense

oligonucleotide having a nucleotide sequence of from about 13 to about 35 nucleotides and which comprises the nucleotide sequence of SEQ ID NO: 5. In another embodiment thereof, the animal is administered an HDAC-1 antisense oligonucleotide having a nucleotide sequence of from about 15 to about 26 nucleotides and which comprises the nucleotide sequence of SEQ ID NO:6. In yet another embodiment thereof, the animal is administered an HDAC-1 antisense oligonucleotide that is SEQ ID NO: 6.

In fourth aspect, the invention provides a method for inhibiting HDAC-7 and/or HDAC-8 activity in a cell, comprising contacting the cell with a small molecule inhibitor of HDAC-7 and/or HDAC-8, wherein HDAC-8 activity is inhibited.

In another embodiment therein, the invention provides a method wherein the inhibition of HDAC-7 and/or HDAC-8 activity in the contacted cell further leads to an inhibition of cell proliferation in the contacted cell. In another embodiment therein, the invention provides a method wherein inhibition of HDAC-7 and/or HDAC-8 activity in the contacted cell further leads to growth retardation of the contacted cell. In another embodiment therein, the invention provides a method wherein inhibition of HDAC-7 and/or HDAC-8 activity in the contacted cell further leads to growth arrest of the contacted cell. In another embodiment therein, the invention provides a method wherein inhibition of HDAC-7 and/or HDAC-8 activity in the contacted cell further leads to programmed cell death of the contacted cell. In another embodiment therein, the invention provides a method wherein inhibition of HDAC-7 and/or HDAC-8 activity in the contacted cell further leads to necrotic cell death of the contacted cell. In another embodiment thereof, the contacted cell is a human cell.

In fifth aspect, the invention provides a method for inhibiting neoplastic cell proliferation in an animal, comprising administering to an animal having at least one neoplastic cell present in its body a therapeutically effective amount of a small molecule

inhibitor of HDAC-7 and/or HDAC-8, whereby neoplastic cell proliferation is inhibited.

In another embodiment thereof, the invention provides a method wherein the animal administered a small molecule inhibitor is a human.

In a sixth aspect, the invention provides a method for inhibiting the induction of cell proliferation, comprising contacting a cell with an antisense oligonucleotide that inhibits the expression of HDAC-7 or HDAC-8 and/or contacting a cell with a small molecule inhibitor of HDAC-7 and/or HDAC-8. In certain preferred embodiments, the cell is a neoplastic cell, and the induction of cell proliferation is tumorigenesis.

In a seventh aspect, the invention provides a method for identifying a small molecule histone deacetylase inhibitor that inhibits the HDAC-7 and/or HDAC-8 isoform, the isoform being required for the induction of cell proliferation. The method comprises contacting the HDAC-7 or HDAC-8 isoform with a candidate small molecule inhibitor and measuring the enzymatic activity of the contacted histone deacetylase isoform, wherein a reduction in the enzymatic activity of the contacted HDAC-7 or HDAC-8 isoform identifies the candidate small molecule inhibitor as a small molecule histone deacetylase inhibitor of the HDAC-7 or HDAC-8 isoform.

In an eighth aspect, the invention provides a method for identifying a small molecule histone deacetylase inhibitor that inhibits HDAC-7 or HDAC-8 isoform, which is involved in the induction of cell proliferation. The method comprises contacting a cell with a candidate small molecule inhibitor and measuring the enzymatic activity of the contacted histone deacetylase isoform, wherein a reduction in the enzymatic activity of the HDAC-7 or HDAC-8 isoform identifies the candidate small molecule inhibitor as a small molecule histone deacetylase inhibitor of HDAC-7 or HDAC-8.

In a ninth aspect, the invention provides a small molecule histone deacetylase inhibitor identified by the method of the seventh or the eighth aspect of the invention. Preferably, the histone deacetylase small molecule inhibitor is substantially pure.

In a tenth aspect, the invention provides a method for inhibiting cell proliferation in a cell comprising, contacting a cell with at least two reagents selected from the group consisting of an antisense oligonucleotide that inhibits expression of HDAC-7 or HDAC-8 isoform, a small molecule histone deacetylase inhibitor that inhibits expression or activity of HDAC-7 and/or HDAC-8 isoform, an antisense oligonucleotide that inhibits expression of the HDAC-1 isoform, a small molecule histone deacetylase inhibitor that inhibits the expression or the activity of the HDAC-1 isoform, an antisense oligonucleotide that inhibits expression of a DNA methyltransferase, and a small molecule DNA methyltransferase inhibitor. In certain embodiments, the inhibition of cell growth of the contacted cell is greater than the inhibition of cell growth of a cell contacted with one or more of the anti-HDAC-7 or anti-HDAC-8 reagents. In certain embodiments, each of the reagents selected from the group is substantially pure. In preferred embodiments, the cell is a neoplastic cell. In yet additional embodiments, the reagents selected from the group are operably associated.

In an eleventh aspect, the invention provides a method of inhibiting neoplastic cell growth, comprising contacting a cell with at least two reagents selected from the group consisting of an antisense oligonucleotide that inhibits expression of HDAC-7 or HDAC-8 isoform, a small molecule histone deacetylase inhibitor that inhibits the expression or the activity of HDAC-7 and/or HDAC-8 isoform, an antisense oligonucleotide that inhibits expression of the HDAC-1 isoform, a small molecule histone deacetylase inhibitor that inhibits expression or activity of the HDAC-1 isoform, an antisense oligonucleotide that inhibits expression of a DNA methyltransferase, and a small molecule DNA methyltransferase inhibitor. In some embodiments, the inhibition of cell

growth of the contacted cell is greater than the inhibition of cell growth of a cell contacted with only one of the reagents. In certain embodiments, each of the reagents selected from the group is substantially pure. In preferred embodiments, the cell is a neoplastic cell. In yet additional preferred embodiments, the reagents selected from the group are operably associated.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig 1 shows that expression of HDAC-7 mRNA was inhibited in a dose-dependent manner by both AS-1 and AS-2 oligos directed against human HDAC7 in human cancer A549 cells.

Fig 2 shows that expression of HDAC-7 protein was inhibited and expression of p21 protein induced by both AS-1 and AS-2 oligos directed against human HDAC7 in human cancer A549 cells.

Fig. 3 shows time course analysis of expression of HDAC-7 mRNA by AS-1 oligo directed against human HDAC7 in human cancer A549 cells.

Fig 4 shows time course analysis of HDAC-7 protein expression by both AS-1 and AS-2 oligos directed against human HDAC7 in human cancer A549 cells.

Fig 5 shows that expression of HDAC-8 mRNA was inhibited in a dose-dependent manner by both AS-1 and AS-2 oligos directed against human HDAC8 in human cancer A549 cells.

Fig. 6 shows time course analysis of expression of HDAC-8 mRNA by AS-2 oligo directed against human HDAC8 in human cancer A549 cells.

Fig 7 shows a growth curve of human cancer A549 cells treated with human HDAC-7.

Fig 8 shows a growth curve of human cancer A549 cells treated with varying dose of human AS-1 or AS-2 oligos directed against human HDAC-8.

Fig 9 shows cell cycle analysis of human A549 cancer cells treated with AS-1, AS-2 or MM-1 oligos directed against human HDAC7.

Fig. 10 shows cell cycle analysis of human A549 cancer cells treated with human HDAC8 antisense inhibitors and oxamflatin.

Fig 11 shows dose-dependent induction of apoptosis of human cancer A549 cells by HDAC-8 and HDAC-1 antisense inhibitors.

Fig 12 shows that HDAC-1 or HDAC-8 antisense inhibitor did not induce apoptosis in human normal epithelial HMEC cells.

Fig. 13 shows that inhibition of HDAC1 expression by its antisense inhibitor leads to apoptosis of human cancer A549 cells but not normal HMEC cells.

Fig. 14 shows induction of apoptosis of human cancer A549 and T24 cells by HDAC-8 and HDAC-1 antisense inhibitors.

Fig. 15 shows time-dependence of apoptosis induction of human cancer A549 cells by HDAC-1 or HDAC-8 antisense or mismatch oligos.

Fig 16 shows co-inhibition of HDAC-1 with HDAC-8, or HDAC-1 with HDAC-7, but not the other combinations, by antisense inhibitors synergized in induction of apoptosis of human cancer A549 cells.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The patent and scientific literature referred to herein establishes knowledge that is available to those with skill in the art. The issued patents, applications, and references, including GenBank database sequences, that are cited herein are hereby incorporated by reference to the same extent as if each was specifically and individually indicated to be incorporated by reference.

The invention provides methods and reagents for modulating histone deacetylase (HDAC) isoforms, particularly HDAC-7 and HDAC-8, by inhibiting expression at the nucleic acid level or by inhibiting enzymatic activity at the protein level. The invention provides for the specific inhibition of specific histone deacetylase isoforms involved in tumorigenesis, and thus provides a treatment for cancer. The invention further provides for the specific inhibition of specific HDAC isoforms involved in cell proliferation and thus provides a treatment for cell proliferative disorders.

The inventors have made the surprising discovery that the specific inhibition of HDAC-7 and 8 dramatically induces apoptosis and growth arrest in cancerous cells. Accordingly, in a first aspect, the invention provides agents that inhibit the activity of the HDAC-7 and HDAC-8 isoforms.

In certain preferred embodiments of the first aspect of the invention, the agent that inhibits the HDAC-7 and HDAC-8 isoforms is an oligonucleotide that inhibits expression of a nucleic acid molecule encoding the HDAC-7 and HDAC-8 isoforms. The nucleic acid molecule encoding the HDAC-7 and HDAC-8 isoforms may be genomic DNA (*e.g.*, a gene), cDNA, or RNA. In some embodiments, the oligonucleotide inhibits transcription of mRNA encoding the HDAC-7 or HDAC-8 isoforms. In other embodiments, the oligonucleotide inhibits translation of the HDAC-7 or HDAC-8 isoforms. In certain embodiments the oligonucleotide causes the degradation of the nucleic acid molecule.

In a preferred embodiment thereof, the agent of the first aspect of the invention is an antisense oligonucleotide complementary to a region of RNA that encodes a portion of HDAC-7 or HDAC-8 or to a region of double-stranded DNA that encodes a portion of HDAC-7 or HDAC-8 isoforms. In one embodiment thereof, the antisense oligonucleotide is a chimeric oligonucleotide. In another embodiment thereof, the antisense oligonucleotide is a hybrid oligonucleotide. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 13 to about 35 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In still yet another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 15 to about 26 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 20 to about 26 nucleotides selected from the nucleotide sequence of SEQ ID NO: 1. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 13 to about 35 nucleotides and which comprises the nucleotide sequence of SEQ ID NO: 2. In still yet another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 15 to about 26 nucleotides and which comprises the nucleotide sequence of SEQ ID NO: 2. In another embodiment thereof, the antisense oligonucleotide has a nucleotide sequence of from about 20 to about 26 nucleotides and which comprises the nucleotide sequence of SEQ ID NO:2. In another embodiment thereof, the antisense oligonucleotide is SEQ ID NO: 3. In another embodiment thereof, the antisense oligonucleotide has one or more phosphorothioate internucleoside linkages. In another embodiment thereof, the antisense oligonucleotide further comprises a length of 20-26 nucleotides. In still another embodiment thereof, the antisense oligonucleotide is modified such that the terminal four nucleotides at the 5' end of the oligonucleotide and the terminal four nucleotides at the 3' end of the oligonucleotide each have 2' -O- methyl groups attached to their sugar residues.

In certain preferred embodiments of the first aspect, the agent that inhibits the HDAC-7 and/or HDAC-8 isoform in a cell is a small molecule inhibitor that inhibits expression of a nucleic acid molecule encoding HDAC-7 or HDAC-8 isoform or activity of the HDAC-7 and/or HDAC-8 protein.

The term "small molecule" as used in reference to the inhibition of histone deacetylase is used to identify a compound having a molecular weight preferably less than 1000 Da, more preferably less than 800 Da, and most preferably less than 600 Da, which is capable of interacting with a histone deacetylase and inhibiting the expression of a nucleic acid molecule encoding an HDAC isoform or activity of an HDAC protein. Inhibiting histone deacetylase enzymatic activity means reducing the ability of a histone deacetylase to remove an acetyl group from a histone. In some preferred embodiments, such reduction of histone deacetylase activity is at least about 50%, more preferably at least about 75%, and still more preferably at least about 90%. In other preferred embodiments, histone deacetylase activity is reduced by at least 95% and more preferably by at least 99%. In a particularly preferred embodiment, the small molecule inhibitor of HDAC is an inhibitor of HDAC-7 and/or HDAC-8.

Preferably, such inhibition is specific, *i.e.*, the histone deacetylase inhibitor reduces the ability of a histone deacetylase to remove an acetyl group from a histone at a concentration that is lower than the concentration of the inhibitor that is required to produce another, unrelated biological effect. Preferably, the concentration of the inhibitor required for histone deacetylase inhibitory activity is at least 2-fold lower, more preferably at least 5-fold lower, even more preferably at least 10-fold lower, and most preferably at least 20-fold lower than the concentration required to produce an unrelated biological effect.

Preferred agents that inhibit HDAC-7 and/or HDAC-8 inhibit growth of human cancer cells, independent of their p53 status. These agents induce apoptosis in cancer cells and cause growth arrest. They also can induce transcription of p21^{WAF1} (a tumor suppressor gene), *Bax*, an extremely important gene involved in apoptosis regulation and *GADD45*, a stress-induced gene and important regulator of cell growth. These agents may exhibit both *in vitro* and *in vivo* anti-tumor activity. Inhibitory agents that achieve one or more of these results are considered within the scope of this aspect of the invention.

The antisense oligonucleotides according to the invention are complementary to a region of RNA or to a region of double-stranded DNA that encodes a portion of one or more histone deacetylase isoforms (taking into account that homology between different isoforms may allow a single antisense oligonucleotide to be complementary to a portion of more than one isoform). For purposes of the invention, the term "oligonucleotide" includes polymers of two or more deoxyribonucleosides, ribonucleosides, or any combination thereof. Preferably, such oligonucleotides have from about 6 to about 50 nucleoside residues, and most preferably from about 12 to about 30 nucleoside residues. The nucleoside residues may be coupled to each other by any of the numerous known internucleoside linkages. Such internucleoside linkages include without limitation phosphorothioate, phosphorodithioate, alkylphosphonate, alkylphosphonothioate, phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate, bridged phosphorothioate, and sulfone internucleotide linkages. These internucleoside linkages preferably are phosphotriester, phosphorothioate, or phosphoramidate linkages, or combinations thereof.

Preferably, the oligonucleotides may also contain 2'-O-substituted ribonucleotides. For purposes of the invention the term "2'-O-substituted" means

substitution of the 2' position of the pentose moiety with an -O-lower alkyl group containing 1-6 saturated or unsaturated carbon atoms, or with an -O-aryl or allyl group having 2-6 carbon atoms, wherein such alkyl, aryl, or allyl group may be unsubstituted or may be substituted, *e.g.*, with halo, hydroxy, trifluoromethyl, cyano, nitro, acyl, acyloxy, alkoxy, carboxyl, carbalkoxyl, or amino groups; or such 2' substitution may be with a hydroxy group (to produce a ribonucleoside), an amino or a halo group, but not with a 2'-H group. The term "alkyl" as employed herein refers to straight and branched chain aliphatic groups having from 1 to 12 carbon atoms, preferably 1-8 carbon atoms, and more preferably 1-6 carbon atoms, which may be optionally substituted with one, two or three substituents. Unless otherwise apparent from context, the term "alkyl" is meant to include saturated, unsaturated, and partially unsaturated aliphatic groups. When unsaturated groups are particularly intended, the terms "alkenyl" or "alkynyl" will be used. When only saturated groups are intended, the term "saturated alkyl" will be used. Preferred saturated alkyl groups include, without limitation, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *sec*-butyl, *tert*-butyl, pentyl, and hexyl.

The term oligonucleotide also encompasses such polymers having chemically modified bases or sugars and/or having additional substituents including, without limitation, lipophilic groups, intercalating agents, diamines, and adamantane. The term oligonucleotide also encompasses such polymers as PNA and LNA.

For purposes of the invention, the term "complementary" means having the ability to hybridize to a genomic region, a gene, or an RNA transcript thereof, under physiological conditions. Such hybridization is ordinarily the result of base-specific hydrogen bonding between complementary strands, preferably to form Watson-Crick or Hoogsteen base pairs, although other modes of hydrogen bonding, as well as base stacking can lead to hybridization. As a practical matter, such hybridization can be

inferred from the observation of specific gene expression inhibition, which may be at the level of transcription or translation (or both).

Particularly preferred antisense oligonucleotides utilized in this aspect of the invention include chimeric oligonucleotides and hybrid oligonucleotides.

For purposes of the invention, a "chimeric oligonucleotide" refers to an oligonucleotide having more than one type of internucleoside linkage. One preferred embodiment of such a chimeric oligonucleotide is a chimeric oligonucleotide comprising internucleoside linkages, phosphorothioate, phosphorodithioate, internucleoside linkages and phosphodiester, preferably comprising from about 2 to about 12 nucleotides. Some useful oligonucleotides of the invention have an alkylphosphonate-linked region and an alkylphosphonothioate region (see *e.g.*, Pederson *et al.* U.S. Patent Nos. 5,635,377 and 5,366,878). Preferably, such chimeric oligonucleotides contain at least three consecutive internucleoside linkages that are phosphodiester and phosphorothioate linkages, or combinations thereof.

For purposes of the invention, a "hybrid oligonucleotide" refers to an oligonucleotide having more than one type of nucleoside. One preferred embodiment of such a hybrid oligonucleotide comprises a ribonucleotide or 2'-O-substituted ribonucleotide region, preferably comprising from about 2 to about 12 2'-O-substituted nucleotides, and a deoxyribonucleotide region. Preferably, such a hybrid oligonucleotide contains at least three consecutive deoxyribonucleosides and contains ribonucleosides, 2'-O-substituted ribonucleosides, or combinations thereof (see *e.g.*, Metelev and Agrawal, U.S. Patents Nos. 5,652,355 and 5,652,356).

The exact nucleotide sequence and chemical structure of an antisense oligonucleotide utilized in the invention can be varied, so long as the oligonucleotide retains its ability to modulate expression of the target sequence, *e.g.*, the HDAC-7 or the

HDAC-8 isoform. This is readily determined by testing whether the particular antisense oligonucleotide is active by quantitating the amount of mRNA encoding the HDAC-7 or the HDAC-8 isoform, quantitating the amount of the HDAC-7 or the HDAC-8 isoform protein, quantitating the the HDAC-7 or the HDAC-8 isoform enzymatic activity, or quantitating the ability of the the HDAC-7 or the HDAC-8 isoform, for example, to inhibit cell growth in a an *in vitro* or *in vivo* cell growth assay, all of which are described in detail in this specification. The term "inhibit expression" and similar terms used herein are intended to encompass any one or more of these parameters.

Antisense oligonucleotides according to the invention may conveniently be synthesized on a suitable solid support using well-known chemical approaches, including H-phosphonate chemistry, phosphoramidite chemistry, or a combination of H-phosphonate chemistry and phosphoramidite chemistry (*i.e.*, H-phosphonate chemistry for some cycles and phosphoramidite chemistry for other cycles). Suitable solid supports include any of the standard solid supports used for solid phase oligonucleotide synthesis, such as controlled-pore glass (CPG) (see, *e.g.*, Pon, R. T., *Meth. Molec. Biol.* **20**:465-496, 1993).

Antisense oligonucleotides according to the invention are useful for a variety of purposes. For example, they can be used as "probes" of the physiological function of specific histone deacetylase isoforms by being used to inhibit the activity of specific histone deacetylase isoforms in an experimental cell culture or animal system and to evaluate the effect of inhibiting such specific histone deacetylase isoform activity. This is accomplished by administering to a cell or an animal an antisense oligonucleotide that inhibits one or more histone deacetylase isoform expression according to the invention and observing any phenotypic effects. In this use, the antisense oligonucleotides used according to the invention are preferable to traditional "gene knockout" approaches

because they are easier to use, and because they can be used to inhibit specific histone deacetylase isoform activity at selected stages of development or differentiation.

Preferred antisense oligonucleotides of the invention inhibit either the transcription of a nucleic acid molecule encoding the the HDAC-7 or the HDAC-8 isoform, and/or the translation of a nucleic acid molecule encoding the the HDAC-7 or the HDAC-8, and/or lead to the degradation of such nucleic acid molecules. HDAC-7- or HDAC-8-encoding nucleic acid molecules may be RNA or double stranded DNA regions and include, without limitation, intronic sequences, untranslated 5' and 3' regions, intron-exon boundaries, as well as coding sequences from the HDAC-7 or the HDAC-8 isoform genes.

Antisense oligonucleotides for human HDAC isotype polynucleotides may be designed from known HDAC isotype sequence data. For example, the following amino acid sequences are available from GenBank for HDAC-7, and HDAC-8: AAF63491, and AAF73076, respectively, and the following nucleotide sequences are available from GenBank for HDAC-7, and HDAC-8: AF239243, and AF230097, respectively.

The sequences encoding histone deacetylases from many non-human animal species are also known. Accordingly, the antisense oligonucleotides of the invention may also be complementary to a region of RNA or to a region of double-stranded DNA that encode the HDAC-7 or the HDAC-8 isoform from non-human animals. Antisense oligonucleotides according to these embodiments are useful as tools in animal models for studying the role of specific histone deacetylase isoforms.

To study the expression pattern of HDAC-1, HDAC-7 and HDAC-8 in various cancer samples, the following analysis was carried out. Briefly, human A549 and/or T24 human bladder carcinoma cells were seeded in 10 cm tissue culture dishes one day prior to oligonucleotide treatment. The cancer cell samples were obtained from

________________and were grown under the recommended culture conditions. Cells were harvested, and total RNAs were analyzed by Northern blot analysis. Briefly, total RNA was extracted using RNeasy miniprep columns (QIAGEN). Ten to twenty μg of total RNA was run on a formaldehyde-containing 1% agarose gel with 0.5 M sodium phosphate (pH 7.0) as the buffer system. RNAs were then transferred to nitrocellulose membranes and hybridized with the indicated radiolabeled DNA probes. Autoradiography was performed using conventional procedures. The results are shown in Table 1 below. These results demonstrate that expression of these HDAC isotypes varies widely among different types of cancer cells and are thus amenable to antisense oligonucleotide analysis of cancer cell phenotype.

Table 1: Human HDAC Isotype mRNA Expression in Paired Normal vs. Tumor Tissues from Patients*

# of patients analysed	Tissue	Expression in normal tissues #			% of patients with altered expression in tumor tissues**					
					HDAC1		HDAC7		HDAC8	
		HDAC1	HDAC7	HDAC8	up	down	up	down	up	down
50	breast	++	++	++	30	32	10	48	10	48
42	uterus	++	++	++	40	2	19	14	19	14
35	colon	++++	++	++	11	43	43	14	46	14
27	stomach	+++	++	++	22	30	30	19	30	19
12	ovary	++	++	++	42	25	8	67	8	67
1	cervix	+	+	+	100	0	0	0	0	0
21	lung	++	++	++	52	10	19	14	19	14
20	kidney	++	++	++	10	70	0	75	0	75
18	rectum	+++	++	++	0	61	39	0	39	0
2	small intestine	+++	++	++	50	0	50	0	50	0
6	thyroid	+++	++	++	17	33	17	0	17	0
4	prostate	+++	++	++	25	0	25	0	25	0
1	pancreas	++++	++++	+++	0	0	0	0	0	0

*Expression was analyzed by Cancer Profiling Array (Clontech); Expression of each HDAC isotype was normalized against that of ubiquitin;
"+" means detectable; "++" means 2-4X over "+"; "+++" means 5-9X over "+"; "++++" means more than 10X over "+".
** Expression of the HDAC isotype in tumor tissues was changed at least 1.5 fold over that in the paired normal tissues.

Particularly, preferred oligonucleotides have nucleotide sequences of from about 13 to about 35 nucleotides which include the nucleotide sequences shown in Table 2 below.

These oligonucleotides have nucleotide sequences of from about 15 to about 26 nucleotides of the nucleotide sequences shown below in Table 2. Most preferably, the oligonucleotides shown below have phosphorothioate backbones, are 20-26 nucleotides in length, and are modified such that the terminal four nucleotides at the 5' end of the oligonucleotide and the terminal four nucleotides at the 3' end of the oligonucleotide each have 2' -O- methyl groups attached to their sugar residues.

Table 2: HDAC isotype-specific antisense and mismatch oligos

Oligo	Target	Accession Number	Nucleotide Position	Sequence	position within Gene
HDAC1 AS1	Human HDAC1	U50079	1585-1604	5'-GAAACGTGAGGGACTCAGCA-3'	3'-UTR
HDAC1 MM1	Human HDAC1	U50079	1585-1604	5'-GUUAGGTGAGGCACTGAGGA-3'	3'-UTR
HDAC1 AS2	Human HDAC1	U50079	1565-1584	5'-GGAAGCCAGAGCTGGAGAGG-3'	3'-UTR
HDAC2 AS	Human HDAC2	U31814	1643-1662	5'-GCUGAGCTGTTCTGATUUGG-3'	3'-UTR
HDAC2 MM	Human HDAC2	U31814	1643-1662	5'-CGUGAGCACTTCTCATUUCC-3'	3'-UTR
HDAC3 AS1	Human HDAC3	AF039703	1276-1295	5'-CGCUTTCCTTGTCATTGACA-3'	3'-UTR
HDAC3 MM1	Human HDAC3	AF039703	1276-1295	5'-GCCUTTCCTACTCATTGUGU-3'	3'-UTR
HDAC3 AS2	Human HDAC3	AF039703	1487-1506	5'-GGUACCATTGTCAGGCCUUG-3'	3'-UTR
HDAC3 MM2	Human HDAC3	AF039703	1487-1506	5'-CCUACCATTCACAGGCCUAC-3'	3'-UTR
HDAC4 AS1	Human HDAC4	AB006626	514-33	5-GCUGCCTGCCGTGCCCACCC-3'	5'-UTR
HDAC4 MM1	Human HDAC4	AB006626	514-33	5'-CGUGCCTGCGCTGCCCACGG-3'	5'-UTR
HDAC4 AS2	Human HDAC4	AB006626	7710-29	5'-UACAGTCCATGCAACCUCCA-3'	3'-UTR
HDAC4 MM2	Human HDAC4	AB006626	7710-29	5'-AUCAGTCCAACCAACCUCGU-3'	3'-UTR
HDAC5 AS1	Human HDAC5	BE794912	1-20	5'-GCAGCGGCGGCAGCACCUCC-3'	5'-UTR
HDAC5 AS2	Human HDAC5	AF039691	2663-2682	5'-CTTCGGTCTCACCTGCTTGG-3'	3'-UTR
HDAC5 AS3	Human HDAC5	BE794912	259-278	5'-CGUUGGGAGAGTTCATGCCG-3'	5'-UTR
HDAC6 AS	Human HDAC6	AJ011972	3791-3810	5'-CAGGCTGGAATGAGCTACAG-3'	3'-UTR
HDAC6 MM	Human HDAC6	AJ011972	3791-3810	5'-GACGCTGCAATCAGGTAGAC-3'	3'-UTR
HDAC7 AS1	Human HDAC7	AF239243	65-84	5'-CAGGCTCACTTGACAAUGGC-3'	5'-UTR
HDAC7 MM1	Human HDAC7	AF239243	65-84	5'-GUGGCACACAAGACAAUCCC-3'	5'-UTR
HDAC7 AS2	Human HDAC7	AF239243	2896-2915	5'-CUUCAGCCAGGATGCCCACA-3'	3'-UTR
HDAC8 AS1	Human HDAC8	AF230097	51-70	5'-CUCCGGCTCCTCCATCUUCC-3'	5'-UTR
HDAC8 MM1	Human HDAC8	AF230097	51-70	5'-GACCGGCTGCACCATCTTGG-3'	5'-UTR
HDAC8 AS2	Human HDAC8	AF230097	1328-1347	5'-AGCCAGCTGCCACTTGAUGC-3'	3'-UTR
HDAC8 MM2	Human HDAC8	AF230097	1328-1347	5'-UCCCAGCTGGCTCTTGAAGG-5'	3'-UTR
UMM*				5'-NNNNNNNNNNNNNNNNNNNN-3'	

* UMM is a second generation phosphorothioate oligo with 4X4 2'-O-methyl modification.

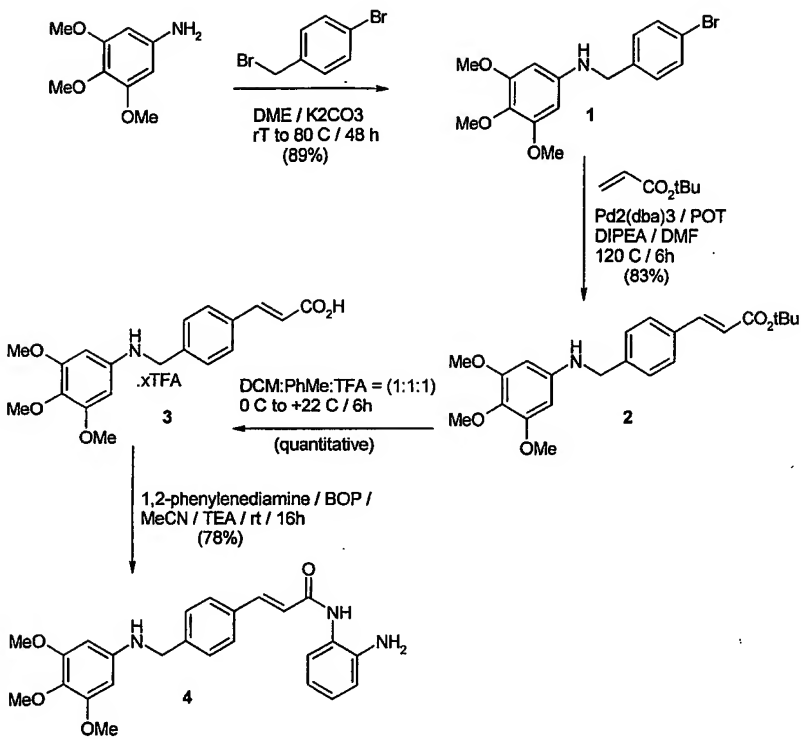
Each nucleotide position (N) contains 25% of dA, 25% of dT, 25% of dG, 25% of dC

The antisense oligonucleotides according to the invention may optionally be formulated with any of the well known pharmaceutically acceptable carriers or diluents (see preparation of pharmaceutically acceptable formulations in, *e.g.*, Remington's Pharmaceutical Sciences, 18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, PA, 1990), with the proviso that such carriers or diluents not affect their ability to modulate HDAC activity.

In certain preferred embodiments, the agent that inhibits the HDAC-7 and/or HDAC-8 isoform is a small molecule. In certain preferred embodiments, the small molecule inhibits the enzymatic activity of the HDAC-7 or HDAC-8 isoform.

Small molecule isotype-specific inhibitors of the invention may be conveniently prepared according to the following schemes or using other art-recognized methods.

N-(2-Aminophenyl)-3-{4-[(3,4,5-trimethoxyphenylamino)-methyl]-phenyl} acrylamide


MeO
NH2
MeO
OMe
Br
Br
DME / K2CO3
rT to 80 C / 48 h
(89%)
H
N
1
CO2tBu
Pd2(dba)3 / POT
DIPEA / DMF
120 C / 6h
(83%)
2
CO2H
.xTFA
3
DCM:PhMe:TFA = (1:1:1)
0 C to +22 C / 6h
(quantitative)
1,2-phenylenediamine / BOP /
MeCN / TEA / rt / 16h
(78%)
O
NH
4

Step 1: (4-Bromobenzyl)-(3,4,5-trimethoxyphenyl)-amine (1)

A mixture of 3,4,5-trimethoxyaniline (10.12g; 55.24 mmol), 4-bromobenzylbromide (7.61g; 30.45 mmol) and K2CO3 (7.4g; 53.6 mmol) was suspended in dry DME (195 mL) and stirred under nitrogen at room temperature for 12 h, then slowly warmed up to + 80° C and stirred at this temperature for one day. The mixture was dilute with EtOAc and washed with saturated NaHCO3 followed by water (X3), dried (MgSO4) filtered and concentrated. The crude oil was crystallized from MeOH to afford 7.59 g (21.55 mmol; 71%). The remaining mother liquors were concentrated and purified by chromatographic column (SiO2; elution hexane:ether = 7:3) to give extra 1.94 g (5.51 mmol, 18%). Overall yield 89%

Step 2: 3-{4-[(3,4,5-Trimethoxyphenylamino)-methyl]-phenyl}-acrylic acid *tert*-butyl ester (2)

A mixture of bromide **1** (6.78g; 19.23 mmol); tri-o-tolylphosphine (735 mg; 2.41 mmol; 12% mol) and Pd2(dba)3 (700 mg; 0.76 mmol; 8% mol) was dissolved in degassed DMF (15 mL) under nitrogen and treated with DIPEA (7 mL) followed by tert-butylacrylate (7 mL; 46.8 mmol; 2.4 eq.). The suspension was placed in an oil bath preheated to +120 C, and stirred at this temperature for 5h under nitrogen (balloon). The excess of DMF was removed under high vacuum and the crude mixture purified through chromatographic column (silicagel; elution 30% to 50% ether in hexane) to afford 6.38g (16.0 mmol; 83%) of pure ester **2**.

Step 3: 3-{4-[(3,4,5-Trimethoxyphenylamino)-methyl]-phenyl}-acrylic acid; compound with trifluoro-acetic acid (3)

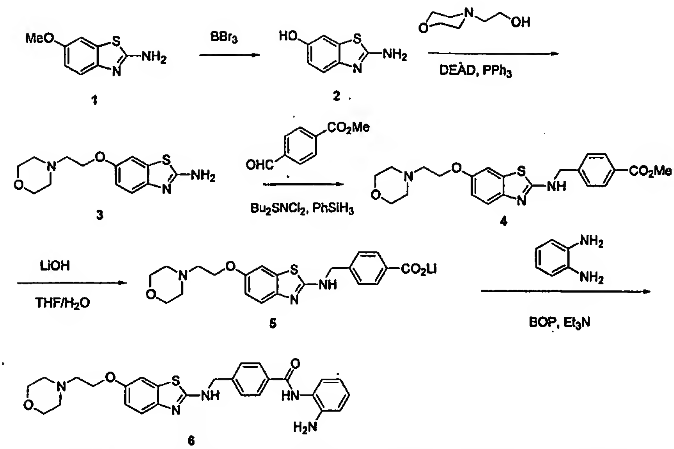
A solution of ester **2** (22.54 g; 56.5 mmol) in 200 mL of DCM: PhMe = 1:1, was cooled down to 0°C and treated with neat, freshly distilled TFA (100 mL), added dropwise from a dropping funnel in a 12 min period with vigorous stirring. The mixture was then

slowly warmed up to + 22 C in a 6h period; concentrated under vacuum and the excess of TFA removed by codistilling with MeCN-PhMe (X3) to give 29.86 g of acid **3** combined with approximately two molecules of TFA. Consistent spectrums.

Step 4: N-(2-Aminophenyl)-3-{4-[(3,4,5-trimethoxyphenylamino)-methyl]-phenyl} acrylamide (4)

A mixture of acid **3** (14 mmol), 1,2-phenylenediamine (1910 mg; 17.66 mmol; 1.26 eq.) and BOP (6876 mg; 15.53 mmol; 1.11 eq.) was dissolved in dry acetonitrile (300 mL), treated with triethylamine (8 mL; 57.6 mmol) and stirred at room temperature for 14h. The solution as concentrated *in vacuo*, diluted with EtOAc, washed with sat. NaHCO3, then water, dried (MgSO4), filtered and concentrated. A solid precipitated took place from a solution of this material in DCM (3.84 g; 8.87 mmol; 63%). The supernatant was concentrated and submitted to chromatographic purification through column on silica gel (elution 5% MeOH in DCM), to afford extra 0.915 g (2.11 mmol; 15% yield). Overall yield: 78%.

1H-NMR (300.07 MHz; DMSO-*d6*) □ (ppm): 9.36 (s; 1H); 7.58 (d; J=8.3; 2H); 7.43 (d; J=7.9; 2H); 7.33 (d; J=7.9; 1H); 6.90 (m, 2H); 6.75 (d; J=7.9; 1H); 6.58 (t; J=5.9; 1H); 5.89 (s; 2H); 4.93 (bs; 2H); 4.27 (d; J=5.7; 2H); 3.65 (s; 6H); 3.58 (s; 3H). HRMS: calc for C25H27N3O4 433.2001; found: 433.2006



N-(2-Amino-phenyl)-4-{[6-(2-morpholin-4-yl-ethoxy)-benzothiazol-2-ylamino]-methyl}-benzamide (6):

Step 1: 2-Amino-benzothiazol-6-ol (2):

A suspension of 2-Amino-6-methoxybenzothiazole (5.00g, 27.8mmol) in DCM (70mL) was cooled to 0°C under N_2. Neat boron tribromide (3.93mL, 41.6mmol) was added dropwise, while stirring. After 1h at 0°C, formation of sticky insoluble material prevented stirring so the bottom of the flask was scraped with a spatula and the insoluble material dispersed using ultrasound bath, and more BBr_3 (1.31mL, 13.9mmol) was added dropwise while stirring. The light yellow mixture was stirred for 3 h, allowing to warm-up slowly from 0°C to 10°C. The reaction was slowly quenched by dropwise addition of methanol until no more heat is evolved, (caution: evolution of hydrogen bromide gas!). After excess methanol is added, the mixture was stirred at r.t. overnight and the white solid is collected by filtration (6.04g, 88% yield). This hydrobromic salt was dissolved in

water and poured into a separating funnel. The aqueous layer was washed with ethyl acetate, and neutralized with a saturated aqueous solution of sodium bicarbonate, while stirring. The resulting white crystals (3.63g, 79% yield) were collected by filtration and dried in the oven at 135°C for 1h.

Step 2: 6-(2-Morpholin-4-yl-ethoxy)-benzothiazol-2-ylamine (3):

To a solution of benzothiazole **2** (3.62g, 21.8mmol) in THF at r.t. under N_2, were successively added neat 4-(2-hydroxyethyl)morpholine (3.17mL, 26.14mmol), triphenylphosphine (7.43g, 28.3mmol). Diethyl azodicarboxylate (4.46mL, 28.3mmol) was added dropwise. The solution was stirred for 3.5h and THF was partially removed *in vacuo*. The mixture was partitioned between ethyl acetate and water. The aqueous layer was extracted with fresh EtOAc and the combined organic layers were extracted with HCl 1N (2X). The combined acidic extracts were basified using a saturated aqueous solution of sodium bicarbonate and the precipitate was taken into fresh ethyl acetate (2X). These combined organic layers were washed with brine, dried over $MgSO_4$, and partially concentrated. A white solid crystallized out upon standing overnight and was filtered off. The filtrate was concentrated to impure light yellow oil (5.83g, 96% yield).

Step 3: 4-{[6-(2-Morpholin-4-yl-ethoxy)-benzothiazol-2-ylamino]-methyl}-benzoic acid methyl ester (4):

To a round-bottom flask containing benzothiazole **3** (5.80g, 20.8 mmol) was added methyl 4-formylbenzoate (5.11g, 31.1mmol), followed by THF (8mL), dibutyltin dichloride (315mg, 1.04mmol) and drop wise addition of phenylsilane (3.24mL, 31.1mmol). The resulting yellow paste was stirred at r.t. under N_2 over 2.5 days but some starting material remained. The mixture was diluted in some ethyl acetate and the white solid was filtered off. The filtrate was partitioned between EtOAc and water. The aqueous layer was extracted with fresh EtOAc and the combined organic layers were washed with HCl 1N (3X). The combined acidic layers were basified using a saturated

aqueous solution of sodium bicarbonate and the precipitate was taken into fresh ethyl acetate (2X). These combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. The resulting crude oil was purified by flash chromatography using $MeOH/CHCl_3$ (10:90) and two fractions were isolated. The least polar being the desired compound (3.69g, 42% yield) and the most polar being the starting benzothiazole **3** (2.11g, 37% recovery) which can be recycled to increase the amount of **4** produced.

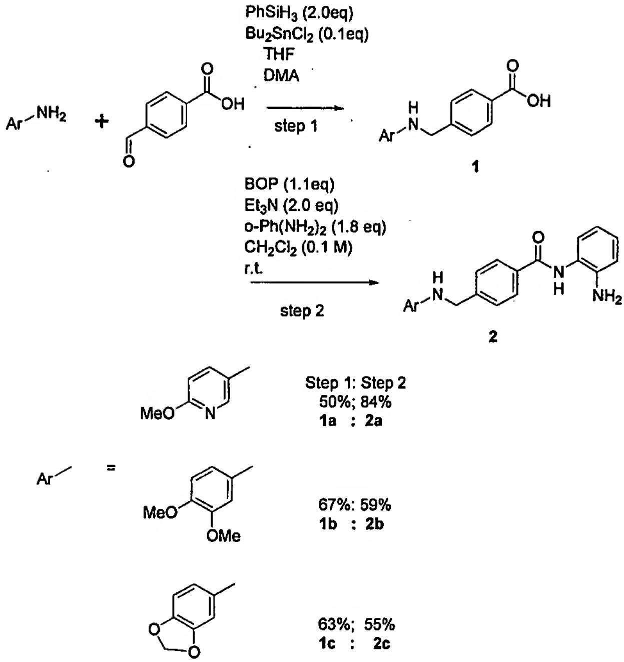
Step 4: 4-{[6-(2-Morpholin-4-yl-ethoxy)-benzothiazol-2-ylamino]-methyl}-benzoic acid lithium salt (5):

Into a solution of ester **4** (4.42g, 11.5mmol) in THF (20mL) at r.t. was poured an aqueous solution (20mL) of lithium hydroxide (1.23g, 51.7mmol). The mixture was stirred at 50°C for 3h and THF was removed *in vacuo* as the lithium carboxylate salt in the remaining water. It was collected by filtration and rinsed with small amount of water. The resulting white paste was dried in the oven at 135°C for 1h yielding the title compound (2.46g, 51%) as a white solid.

Step 5: N-(2-Amino-phenyl)-4-{[6-(2-morpholin-4-yl-ethoxy)-benzothiazol-2-ylamino]-methyl}-benzamide (6):

To a solution of lithium salt **5** (1.75g, 4.17mmol) in DMF (20mL) at r.t. under N_2 was added triethylamine (604□L, 4.34mmol). A solution of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 2.03g, 4.59mmol) in DMF (10mL) was transferred *via canula* into the solution of **5**. The mixture was stirred for 1h and a solution of 1,2-phenylenediamine (498mg, 4.59mmol) in DMF (10mL) was transferred *via canula*, followed by addition of triethylamine (1.74mL, 12.5mmol). The brown mixture was stirred for 2h and DMF was concentrated *in vacuo* at 80°C. The residue was partitioned between ethyl acetate and water. The aqueous layer was extracted with fresh EtOAc and the combined organic layers were washed with HCl 1N (3X). The combined acidic layers were basified using a saturated aqueous solution of

sodium bicarbonate and the precipitate was taken into fresh ethyl acetate (2X). These combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. The resulting foam was dissolved in a minimum premixed methanol/dichloromethane (5:95) started to crystallize immediately. The crystals were collected by filtration and rinsed with dichloromethane. The mother liquors were concentrated and crystallized a second time. The white crystals were combined (958mg, 46%).^{1}H NMR: (Acetone-d_6) δ(ppm): 9.12 (bs, 1H), 8.08 (d, J=8.2 Hz, 2H), 7.65 (d, J=8.5 Hz, 2H), 7.41 (d, J=8.8 Hz, 2H), 7.36 (d, J=2.5 Hz, 1H), 7.08 (t, J=7.1 Hz, 1H), 6.95 (t, J=8.8 Hz, 2H), 6.75 (t, J=7.7 Hz, 1H), 4.85 (s, 2H), 4.71 (bs, 1H), 4.20 (t, J= 6.0 Hz, 2H), 3.70-3.67 (m, 4H), 2.81 (t, J=5.8 Hz, 2H), 2.61-2.58 (m, 4H).



Step 1: 4-[(3,4-Dimethoxyphenylamino)-methyl]-benzoic acid (1b)

In a 50 ml flask, a mixture of 4-aminoveratrole (1.53 g, 10 mmol), 4-formyl-benzoic acid (1.50 g, 10 mmol), dibutyltin dichloride (304 mg, 1 mmol), phenylsilane (2.47 ml, 20 mmol) in anhydrous THF (10 m) and DMA (10 ml) was stirred at rt for overnight. After solvents removal, the crude residue was dissolved in EtOAc (100 ml) and then washed with saturated aqueous solution of $NaHCO_3$ (50 ml x 3). The combined aqueous layers were acidified with

6% of $NaHSO_4$ to pH = 4. The resulting white suspension was filtrated and then the filter cake was washed with water (5 ml x 3). The cake was dried over freeze dryer to afford acid (1.92 g, 67 %) white solid product.

LRMS = 288 (M+1).

Step 2: N-(2-Aminophenyl)-4-[(3,4-dimethoxyphenylamino)-methyl]-benzamide (2b)

In a 150 ml flask, a mixture of acid (1.92 g, 6.69 mmol), benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 3.26 g, 7.37 mmol), triethylamine (1.87 ml, 13.4 mmol), *o*-phenylenediamine (1.30g, 12.02 mmol) in methylenechloride (67 ml) was stirred at rt for 2 h. After solvents removal, the crude residue was dissolved in EtOAc (100 ml) and then washed with $NaHCO_3$ saturated solution and brine 50 ml. The combined organic layers were dried over Na_2SO_4 and the filtrate was concentrated to dryness. The crude material was submitted to a chromatographic purification (column silica, 55%-70 % EtOAc in 1% Et_3N of hexanes) and then the all interested fractions were concentrated to dryness. The residue was suspended in minimum quantities of EtOAc and then filtered to afford final product (1.49 g, 59 %).

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.65 (s, 1H), 7.98 (d, J = 7.9 Hz, 2H), 7.54 (d, J = 7.9 Hz, 2H), 7.22 (d, J = 7.9 Hz, 1H), 7.02 (dd, J = 7.9, 7.9 Hz, 1H), 6.83 (d, J = 7.9 Hz, 1H), 6.72 (d, J = 8.79 Hz, 1H), 6.45 (dd, J = 7.5, 7.5 Hz, 1H), 6.39 (d, J = 2.2 Hz, 1H), 6.01-6.08 (m, 2H), 4.94 (s, 2H, NH_2), 4.36 (d, J = 6.16 Hz, 2H), 3.72 (s, 3H), 3.65 (s, 3H).

N-(2-Aminophenyl)-4-[(6-methoxypyridin-3-ylamino)-methyl]-benzamide (2a)

Following the procedure described in preparation of **2b** as above mentioned but substituting **1a** for **1b** the title compound was obtained in 42 % overall yield.

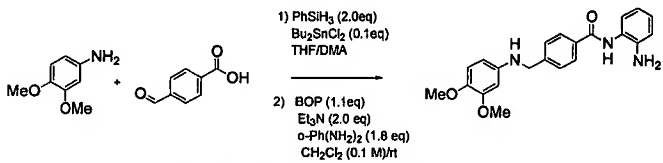
^{1}H NMR (300 MHz, DMSO-D_6) δ (ppm): 9.66 (s, 1H), 7.99 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 7.9 Hz, 2H), 7.50 (d, J = 2.6 Hz, 1H), 7.21 (d, J = 7.5 Hz, 7.9 Hz, 1H), 7.12 (dd, J = 3.08 Hz,

8.79 Hz, 1H), 7.02 (dd, J = 7.0 Hz, 7.5 Hz, 1H), 6.83 (d, J = 7.0 Hz, 1H), 6.65 (m, 2H), 6.15 (t, J = 6.16 Hz, 1H), 4.94 (s, 2H, NH_2), 4.39 (d, J = 6.15 Hz, 2H), 3.75 (s, 3H).

N-(2-Aminophenyl)-4-benzo[1,3]-dioxol-5-ylaminomethyl)-benzamide (2c)

Following the procedure described in in preparation of **2b** as above mentioned but substituting **1c** for **1b** the title compound was obtained in 35 % overall yield

^1H NMR (300 MHz, DMSO-D_6) δ (ppm): 9.65 (s, 1H), 7.98 (d, J = 7.9 Hz, 2H), 7.52 (d, J = 7.9 Hz, 2H), 7.21 (d, J = 7.5 Hz, 1H), 7.02 (dd, J = 7.0, 7.0 Hz, 1H), 6.83 (d, J = 7.5 Hz, 1H), 6.63-6.69 (m, 2H), 6.33 (d, J = 2.2 Hz, 1H), 6.15 (t, J = 6.16 Hz, 1H), 6.04 (dd, J = 2.2, 8.4 Hz, 1H), 5.86 (s, 2H), 4.94 (s, 2H, NH_2), 4.35 (d, J = 6.16 Hz, 2H).



Step 1: 4-[(3,4-Dimethoxyphenylamino)-methyl]-benzoic acid

In a 50 ml flask, a mixture of 4-aminoveratrole (1.53 g, 10 mmol), 4-formyl-benzoic acid (1.50 g, 10 mmol), dibutyltin dichloride (304 mg, 1 mmol), phenylsilane (2.47 ml, 20 mmol) in anhydrous THF (10 m) and DMA (10 ml) was stirred at r.t. overnight. After solvents removal, the crude residue was dissolved in EtOAc (100 ml) and then washed with saturated aqueous solution of $NaHCO_3$ (50 ml x 3) . The combined aqueous layer was acidified with 6% of $NaHSO_4$ to pH = 4. The resulting white suspension was filtrated and then the filter cake was washed with water (5 ml x 3). The cake was dried over freeze dryer to afford acid (1.92 g, 67 %) white solid product.

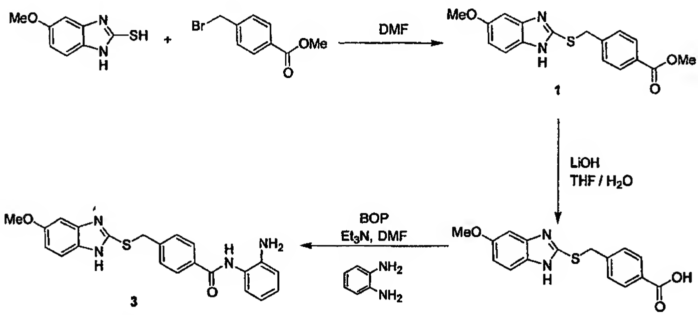
LRMS = 288 (M+1).

Step 2: N-(2-Aminophenyl)-4-[(3,4-dimethoxyphenylamino)-methyl]-benzamide

In a 150 ml flask, a mixture of acid (1.92 g, 6.69 mmol), benzotriazol-1-yloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP, 3.26 g, 7.37 mmol), triethylamine (1.87 ml, 13.4 mmol), *o*-phenylenediamine (1.30g, 12.02 mmol) in methylenechloride (67 ml) was stirred at r.t. for 2 h. After solvents removal, the crude residue was dissolved in EtOAc (100 ml) and then washed with $NaHCO_3$ saturated solution and brine 50 ml. The combined organic layers were dried over Na_2SO_4 and the filtrate was concentrated to dryness. The crude material was submitted to a chromatographic purification (column silica, 55%-70 % EtOAc in 1% Et_3N of hexanes) and then the all interested fractions were concentrated to dryness. The residue was suspended in minimum quantities of EtOAc and then filtered to afford final product (1.49 g, 59 %).

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.65 (s, 1H), 7.98 (d, J = 7.9 Hz, 2H), 7.54 (d, J = 7.9 Hz, 2H), 7.22 (d, J = 7.9 Hz, 1H), 7.02 (dd, J = 7.9 Hz, 7.9 Hz, 1H), 6.83 (d, J = 7.9 Hz, 1H), 6.72 (d, J = 8.79 Hz, 1H), 6.45 (dd, J = 7.49 Hz, 7.49 Hz, 1H), 6.39 (d, J = 2.2 Hz, 1H), 6.01-6.08 (m, 2H), 4.94 (s, 2H, NH_2), 4.36 (d, J = 6.16 Hz, 2H), 3.72 (s, 3H), 3.65 (s, 3H).

15 N-(2-Aminophenyl)-4-(5-methoxy-1H-benzimidazol-2-yl-sulfanylmethyl)-benzamide (3)



Step 1: Methyl-4-(5-methoxy-1H-benzimidazol-2-yl-sulfanylmethyl)-benzoate (1)

To a solution 5-methoxy-2-thiobenzimidazole (2.00 g, 11.1 mmol of in anhydrous DMF (40 ml) was added methy-4-(bromomethyl)-benzoate (2.54 g, 11.1 mmol). The reaction mixture was stirred 16 h at room temperature. The DMF was evaporated and the residue.was triturated in AcOEt during 30 min and then filtered and dried. The desired compound was isolated as the HBr salt: 98% yield, (4.44 g).

^{1}H NMR: (DMSO) δ (ppm): 7.90 (d, J = 8.8 Hz, 2H), 7.56-7.52 (m, 3H), 7.09 (d, J = 2.2 Hz, 1H), 7.01 (dd, J = 8.8 , 2.2 Hz, 1H), 4.73 (s, 2H), 3.82 (s, 6H).

MS: (calc.) 328.1, (obt.), 329.2 (MH)+.

Step 2: 4-(5-Methoxy-1H-benzimidazol-2-yl-sulfanylmethyl)-benzoic acid (2)

A solution of LiOH•H2O (1.02 g, 24.4 mmol) in water (15 ml) was added to a suspension of 1 (3.99 g, 9.75 mmol of in THF (10 ml). The reaction mixture was stirred 16 h at room temperature. The reaction mixture was acidified with a solution of HCl 1 M to pH 4. The desired product was triturated 20 min. at 0°C and then filtered and dried. A white powder was obtained: 100% yield (3.05 g).

^{1}H NMR: (DMSO) δ (ppm): 12.85 (bs, 1H), 7.86 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 8.1 Hz, 2H), 7.35 (d, J = 8.1 Hz, 1H), 6.97 (d, J = 2.2 Hz, 1H), 6.76 (dd, J = 8.8 , 2.2 Hz, 1H), 4.60 (s, 2H), 3.82 (s, 3 H).

MS: (calc.) 314.1, (obt.), 315.1 (MH)+.

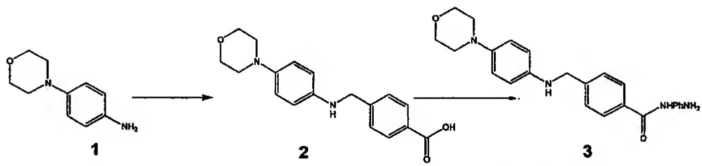
Step 3: N-(2-Amino-phenyl)-4-(5-methoxy-1H-benzimidazol-2-yl-sulfanylmethyl)-benzamide (3)

Following the procedure described in Example 1 step 5 but substituting **4-(5-Methoxy-1H-benzimidazol-2-yl-sulfanylmethyl)-benzoic acid 2** for 7 the title compound 3 was obtained

as a white powder.: 36% yield (933 mg).

^{1}H NMR: (DMSO) δ (ppm): 12.42 (bs, 1H), 9.57 (bs, 1H), 7.89 (d, J = 8.1 Hz, 2H), 7.55 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.8 Hz, 1H), 7.14 (d, J = 7.3 Hz, 1H), 6.98-6.93 (m, 2H), 6.77-6.55 (m, 2H), 6.58 (dd, J = 7.3, 7.3 Hz, 1H), 4.87 (s, 2H), 4.59 (s, 2H), 3.77 (s, 3 H).

MS: (calc.) 404.1, (obt.), 405.4 (MH)+.



N-(2-Amino-phenyl)-4-[(4-morpholin-4-yl-phenylamino)-methyl]-benzamide (3)

Step 1: 4-[(4-Morpholin-4-yl-phenylamino)-methyl]-benzoic acid (2).

A suspension of 4-formylbenzoic acid (2.53g; 16.8 mmol; 1 eq), 4-morpholinoaniline (3g; 16.8 mmol; 1 eq) and Bu_2SnCl_2 (510 mg; 1.68 mmol; 0.1 eq) in dry THF (20 ml) was treated with $PhSiH_3$ (3.31ml; 16.8 mmol; 1 eq) at room temperature for 12 h. The reaction was filtered and the solid product was washed with MeOH. The yield of the reaction was 5.25g (99%).

^{1}H-NMR (300.07 MHz; DMSO-*d6*) □ (ppm):

LRMS: calc 312.37; found: 313.2.

Step 2: N-(2-Amino-phenyl)-4-[(4-morpholin-4-yl-phenylamino)-methyl]-benzamide (3)

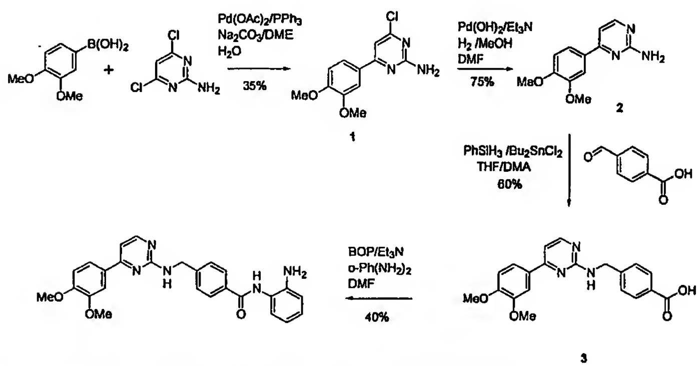
To a solution of acid **2** (2.61g; 8.36 mmol; 1 eq), 1,2-phenylenediamine (903 mg; 8.36 mmol; 1 eq) and BOP (3.70g; 8.36 mmol; 1 eq) in dry DMF (20 ml) was added Et_3N

(4.64ml; 33.4 mmol; 4 eq). After stirring overnight most of the DMF was removed under reduced pressure and chromatographed (Hex:EtAcO: 1:2/ EtAcO). The crystal was obtained in 70% (2.35g).

^{1}H-NMR (300.07 MHz; DMSO-*d6*) □ (ppm): 9.65 (s; 1H); 7.97 (d; J=7.9; 2H); 7.53 (d; J=7.9; 2H); 7.22 (d; J=7.5; 1H); 7.03 (dd, J=7.0, 7.5; 1H); 6.83 (d; J=7.9; 1H); 6.77 (d; J=8.8; 2H); 6.65 (dd; J=7.5, 7.0; 1H); 6.57 (d; J=8.8; 2H); 4.93 (bs; 2H); 4.36 (d; J=5.7; 2H); 3.75 (m; 4H); 2.93 (m; 4H).

LRMS: calc 402.49; found: 403.4.

N-(2-Amino-phenyl)-4-{[4-(3,4-dimethoxy-phenyl)-pyrimidin-2-ylamino]-methyl}-benzamide



Step 1: 4-Chloro-6-(3,4-dimethoxy-phenyl)-pyrimidin-2-ylamine (1)

In a 250ml flask, a mixture of 3,4-dimethoxybenzeneboronic acid (1.12g, 6.15mmol), 2-amino-4,6-dichloro-pyrimidine (2.0g, 12.2mmol), palladium diacetate (0.276g, 1.22mmol), and triphenylphosphine (0.648g, 2.47mmol) were suspended in anhydrous DME (120ml)

under N_2 atmosphere. A solution of Na_2CO_3 (4.06g, 38mmol) in minimum quantities of H_2O (18ml) was added to the mixture. The reflux condenser was applied and the mixture was heated to reflux overnight. The reaction mixture was concentrated to dryness and then purified by flash chromatography (silica gel, 25%-35% EtOAc in 1% Et_3N of hexanes) to give compound **1** (0.64 g, 39%) as a pale yellow solid. LRMS 266 (M+1).

Step 2: 4-(3,4-Dimethoxy-phenyl)-pyrimidin-2-ylamine (2)

In a 50ml flask, compound **1** (0.55g, 2.07 mmol) was dissolved in a mixture of MeOH (10ml) and DMF (10ml) under N_2 atmophere. Triethylamine(0.6ml, 4.3mmol) and palladium hydroxide (0.4g, 20% wt.% Pd on carbon) were added in turn. A H_2 balloon was then applied and the mixture was stirred overnight at rt. The mixture was evaporated to dryness. The residue was dissolved in EtOAc (200ml) and then washed with a saturated solution of $NaHCO_3$ (50ml x 2) and brine (50ml). The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness to give compound **2** (0.424g, 75%) as a off-white solid. LRMS 232 (M+1).

4-{[4-(3,4-Dimethoxy-phenyl)-pyrimidin-2-ylamino]-methyl}-benzoic acid

In a 50 ml flask, a mixture of compound **2** (0.424g, 1.83mmol), 4-formyl-benzoic acid (0.262g, 1.74mmol), dibutyl tin dichloride (35 mg, 0.174mmol), phenyl silane (0.429ml, 3.48mmol) in anhydrous THF (1.83ml) and DMA (1.83ml) was stirred at r.t. overnight. After solvents removal, the crude residue was dissolved in EtOAc (100 ml) and then washed with saturated aqueous solution of $NaHCO_3$ (50 ml x 3). The combined aqueous layer was acidified with 6% of $NaHSO_4$ to pH = 3-4. The resulting white suspension was filtrated and then the filter cake was washed with water (5 ml x 3). The cake was dried over freeze dryer to afford acid **3** (0.4 g, 60 %) white solid product. LRMS = 366 (M+1).

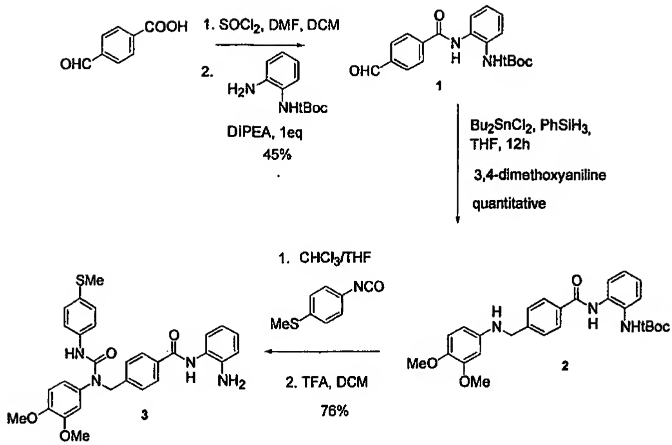
N-(2-Amino-phenyl)-4-{[4-(3,4-dimethoxy-phenyl)-pyrimidin-2-ylamino]-methyl}-

benzamide

In a 50 ml flask, a mixture of compound 3 (400mg, 1.10 mmol), benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP, 581mg, 1.31mmol), triethylamine (0.31ml, 2.19mmol), *o*-phenylenediamine (0.237mg, 2.19mmol) in anhydrous DMF (11ml) was stirred at rt for 2 h. After solvents removal, the residue was dissolved in EtOAc (150ml) and then washed with a saturated solution of $NaHCO_3$ (50ml x 3) and brine (50ml). The combined organic layers were dried over Na_2SO_4 and the filtrate was concentrated to dryness. The crude material was recrystallized in EtOAc to give the title product **4** (200mg, 40%) as a off-white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.64 (s, 1H), 8.35 (d, J = 4.8 Hz, 1H), 7.97 (d, J = 7.9 Hz, 2H), 7.89 (m, 1H), 7.72 (m, 2H), 7.55 (d, J = 7.5 Hz, 2H), 7.2 (d, J = 5.3 Hz, 2H), 7.10 (d, J = 8.4 Hz, 1H), 7.01 (m, 1H), 6.82 (d, J = 7.0 Hz, 1H), 6.41 (t, J = 7.5 Hz, 1 H), 4.92 (s, 2H, NH_2), 4.68 (d, J = 6.16 Hz, 2H), 3.82 (s, 6H)

N-(2-Aminophenyl)-4-[1-(3,4-dimethoxyphenyl)-3-(4-methylsulfanylphenyl)-ureidomethyl]-benzamide



1) Step 1: [2-(4-Formyl-benzoylamino)-phenyl]-carbamic acid *tert*-butyl ester 1

To a suspension of 4-carboxybenzaldehyde (6 g, 40 mmol) in dichloromethane (10 mL) was added thionyl chloride (4.1 mL, 56 mmol, 1.4 eq), followed by DMF (1 mL) dropwise. The mixture was refluxed for 4 hours and excess of thionyl chloride and DMF were removed under reduced pressure. To a solution of (2-aminophenyl)-carbamic acid *tert*-butyl ester (8.32 g, 40 mmol, 1 eq) in dichloromethane (80 mL), stirred at 0°C, was added a suspension of 4-formyl benzoyl chloride in dichloromethane (20 mL), followed by diisopropyl ethylamine (3.61 mL, 20 mmol, 1 eq). The mixture was stirred for 30 minutes at 0°C then at room temperature for 30 minutes. The crude residue was diluted with dichloromethane (300 mL) and washed with water. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under vacuo. The crude residue was purified by column chromatography on silica gel (elution 20% ethyl acetate in hexane) to give 6.1 g (45% yield) of anilide 1. ^1H NMR ($CDCl_3$): □ 10.18 (s, 1H), 9.64 (brs, 1H), 8.20 (d, J = 7.9 Hz, 2H),

8.06 (d, J = 7.9 Hz, 2H), 7.96 (d, J = 7.9 Hz, 1H), 7.28-7.38 (m, 1H), 7.24 (d, J = 4.4 Hz, 1H), 6.84 (s, 1H), 6.81 (d, J = 8.8 Hz, 1H), 1.58 (s, 9H).

2) Step 2: (2-{4-[(3,4-Dimethoxyphenylamino)-methyl]-benzoylamino}-phenyl)-carbamic acid *tert*-butyl ester **2**

To dissolve 3, 4-dimethoxyaniline (1.35 g, 8.82 mmol), 4-formyl-anilide **1** (3 g, 8.82 mmol, 1eq) and tin catalyst (267 mg, 0.88 mmol, 10 mol%) in THF (10 mL). The mixture was stirred at room temperature under nitrogen and then phenylsilane (1,2 mL, 9.7 mmol, 1.1 eq) was slowly added. After stirring overnight at room temperature, THF was removed under reduced pressure. The crude residue was purified by column chromatography on silica gel (elution 40% ethyl acetate in hexane) to give 4.2 g (quantitative yield) of compound **2**. ^{1}H NMR ($CDCl_3$): □ 9.21 (brs, 1H), 8.01 (d, J = 7.9 Hz, 2H), 7.86 (d, J = 7.0 Hz, 1H), 7.55 (d, J = 8.3 Hz, 2H), 7.20-7.34 (m, 3H), 6.89 (brs, 1H), 6.81 (d, J = 8.8 Hz, 1H), 6.37 (d, J = 2.2 Hz, 1H), 6.23 (dd, J = 2.6, 8.3 Hz, 1H), 4.45 (s, 2H), 3.89 (s, 3H), 3.88 (s, 3H), 1.58 (s, 9H).

Step 3: (2-{4-[1-(3,4-Dimethoxyphenyl)-3-(4-methylsulfanylphenyl)-ureidomethyl]-benzoylamino}-phenyl)-carbamic acid *tert*-butyl ester

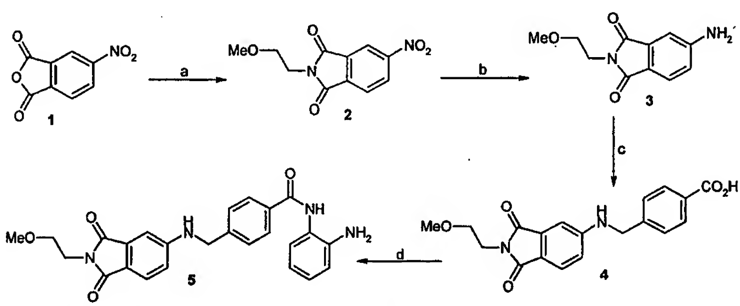
To a solution of anilide **2** (500 mg, 1.047 mmol) in chloroform/THF (1:1, 10 mL) was added isocyanate (169 □L, 1.205 mmol, 1.15 eq). The mixture was stirred overnight at room temperature under nitrogen and the crude residue was concentrated and purified by column chromatography on silica gel (elution 40% ethyl acetate in hexane) to give 606 mg (90% yield) of the title compound. ^{1}H NMR ($CDCl_3$): □ 9.25 (s, 1H), 7.96 (d, J = 8.3 Hz, 2H), 7.85 (d, J = 7.0 Hz, 1H), 7.44 (d, J = 8.3 Hz, 2H), 7.20-7.36 (m, 6H), 6.93 (d, J = 3.5 Hz, 1H), 6.90 (s, 1H), 6.75 (dd, J = 2.2, 8.3 Hz, 1H), 6.68 (dd, J = 2.6 Hz, 1H), 6.33 (s, 1H), 5.0 (s, 2H), 3.97 (s, 3H), 3.85 (s, 3H), 2.51 (s, 3H), 1.57 (s, 9H).

Step 4: *N*-(2-Aminophenyl)-4-[1-(3,4-dimethoxyphenyl)-3-(4-methylsulfanylphenyl)-

ureidomethyl]-benzamide 3

To a solution of the previously described anilide (606 mg, 0.943 mmol) in dichloromethane (10 mL) was added dropwise trifluoroacetic acid (1 mL). The mixture was stirred at room temperature under nitrogen for 1 hour and then was quenched by adding slowly a solution of sodium bicarbonate until neutralization. The crude residue was diluted with dichloromethane and washed with water. The combined organic layers were dried ($MgSO_4$), filtered and concentrated under vacuo. The crude residue was purified by column chromatography on silica gel (elution 30% ethyl acetate in hexane) to give 430 mg (85% yield) of compound **3**. ^{1}H NMR (DMSO-d_6): □ 10.14 (brs, 1H), 7.99 (d, J = 7.9 Hz, 2H), 7.93 (s, 1H), 7.49 (d, J = 8.35 Hz, 4H), 7.39 (d, J = 7.5 Hz, 1H), 7.10-7.30 (2m, 5H), 6.97 (dd, J = 2.2, 8.35 Hz, 1H), 6.77 (dd, J = 2.2, 8.35 Hz, 1H), 5.02 (s, 2H), 3.80 (s, 3H), 3.77 (s, 3H), 2.48 (s, 3H).

3) **N-(2-Amino-phenyl)-4-{[2-(2-methoxy-ethyl)-1,3-dioxo-2,3-dihydro-1H-isoindol-5-ylamino]-methyl}-benzamide**



a. O-Methylethanolamine / PhMe / DMAP / TEA then HOAc / 80C (50%) b. H2 / 10% Pd /C / EtOAc (83%)
c. 4-formylbenzoic acid / PhSiH3 / Bu2SnCl2 / DME, (98%). d. 1,2-phenylenediamine / BOP / DMF /TEA, (55%)

Step 1. Synthesis of 2-(2-Methoxy-ethyl)-5-nitro-isoindole-1,3-dione (2)

A solution of 5-Nitro-isobenzofuran-1,3-dione (1) (654 mg; 3.4 mmol) and DMAP (127 mg) in dichloromethane (15 mL) was treated with O-methylethanolamine (0.60 mL; 6.8 mmol; 2 eq.) and TEA (0.5 mL) and stirred at room temperature for 4h. The reaction mixture was diluted with DCM (150 mL), washed with 10% KHSO4, then brine, dried (MgSO4), filtered and concentrated. The residue was dissolved in HOAc (10 mL) and stirred at 80C for 60 min; concentrated, dissolved in EtOAc (200 mL) and washed with saturated NaHCO3. The organic layer was dried (MgSO4), filtered and concentrated to yield a yellow solid characterized as compound **2**, (425 mg; 1.69 mmol; 50%)

Step 2. Synthesis of 5-Amino-2-(2-methoxy-ethyl)-isoindole-1,3-dione (3)

A solution of **2** (425 mg; 1.69 mmol) in EtOAc (15 mL) was stirred in the presence of 10% Pd/C under H2 (balloon) for 120 min; filtered and concentrated, to give 309 mg (1.4 mmol; 83%) of amine **3**.

Step 3. Synthesis of 4-{[2-(2-Methoxy-ethyl)-1,3-dioxo-2,3-dihydro-1H-isoindol-5-ylamino]-methyl}-benzoic acid (4).

A suspention of amine **3** (309 mg; 1.40 mmol); 4-formylbenzoic acid (215 mg; 1.43 mmol) and Bu2SnCl2 (91 mg; 0.3 mmol; 21%/ mol) in DME (2mL) was treated with PhSiH3 (0.29 mL; 2.28 mL; 1.6 eq) and the mixture stirred overnight at room temperature; the excess of silane quenched with methanol (stirring for 4h); concentrated and purified by chromatographic column on silica gel (elution 5% MeOH in DCM) to give 495 mg (1.39 mmol; 98%) of acid 4.

4) **Step 4. N-(2-Amino-phenyl)-4-{[2-(2-methoxy-ethyl)-1,3-dioxo-2,3-dihydro-1H-isoindol-5-ylamino]-methyl}-benzamide (5)**

A solution of acid **4** (495 mg; 1.39 mmol); 1,2-phenylenediamine (216 mg; 2.0 mmol; 1.4 eq) and BOP (723 mg; 1.64 mmol; 1.2 eq) in DMF (3.0 mL) was treated with TEA (0.6 mL) and stirred at room temperature under nitrogen for 12h; concentrated and purified by chromatographic column on silica gel (elution 5% MeOH in DCM), then triturated with EtOAc and filtered to give 340 mg (0.76 mmol; 55% yield) of pure product 5. From the filtrate, extra 83 mg of product were recovered (purity 89% by HPLC).

1H-NMR (300.07 MHz; DMSO-*d6*) □ (ppm): 9.61 (s; 1H); 7.95 (d; J=7.9; 2H); 7.73 (t; J=5.7; 1H); 7.52 (d; J=8.4; 1H); 7.47 (d, J=7.9; 2H); 7.15 (d, J=7.9; 1H); 6.97 (t; J=7.5; 1H); 6.92 (bs; 1H); 6.86 (d; J=8.4; 1H); 6.77 (d; J=7.9, 1H); 6.59 (t; J=7.5; 1H); 4.89 (bs; 2H); 4.54 (d; J=5.7; 2H); 3.65 (t; J=5.3; 2H); 3.47 (t; J=5.3; 2H); 3.20 (s; 3H).

LRMS: calc for $C_{25}H_{24}N_4O_4$: 444.49; found : 445.4 (M+H).

The reagents according to the invention are useful as analytical tools and as therapeutic tools, including gene therapy tools. The invention also provides methods and compositions which may be manipulated and fine-tuned to fit the condition(s) to be treated while producing fewer side effects.

The invention also provides methods for inhibiting HDAC-7 and/or 8 activity in a cell, comprising contacting the cell with a specific inhibitor of HDAC-7 and/or 8, whereby HDAC-7 and/or HDAC-8 activity is inhibited. As used herein, the term "specific inhibitor" means any molecule or compound that decreases the amount of HDAC-7 or HDAC-8 RNA, HDAC-7 or HDAC-8 protein, and/or HDAC-7 or HDAC-8 protein activity in a cell, relative to other isoforms of HDAC. In an embodiment thereof, the invention provides a method for inhibiting the HDAC-7 or HDAC -8 isoform in a cell comprising contacting the cell with an antisense oligonucleotide of the first aspect of the invention. Preferably, cell proliferation is inhibited in the contacted cell. In preferred embodiments, the cell is a neoplastic cell which may be in an animal, including a human,

and which may be in a neoplastic growth. In certain preferred embodiments, the method of the second aspect of the invention further comprises contacting the cell with a HDAC-7 and/or HDAC-8 small molecule inhibitor that interacts with and reduces the enzymatic activity of the HDAC-7 and/or 8 isoform. In some embodiments, the histone deacetylase small molecule inhibitor is operably associated with the antisense oligonucleotide.

Thus, the antisense oligonucleotides according to the invention are useful in therapeutic approaches to human diseases, including benign and malignant neoplasms, by inhibiting cell proliferation in cells contacted with the antisense oligonucleotides. The phrase "inhibiting cell proliferation" is used to denote an ability of an HDAC-7 or HDAC-8 antisense oligonucleotide or a small molecule HDAC-7 and/or HDAC-8 inhibitor (or combination thereof) to retard the growth of cells contacted with the oligonucleotide or small molecule inhibitor, as compared to cells not contacted.

An assessment of cell proliferation can be made by counting cells that have been contacted with the oligonucleotide or small molecule of the invention and compare that number with the number of non-contacted cells using a Coulter Cell Counter (Coulter, Miami, FL) or a hemacytometer. Where the cells are in a solid growth (*e.g.*, a solid tumor or organ), such an assessment of cell proliferation can be made by measuring the growth of the tissue or organ with calipers, and comparing the size of the growth of contacted cells with non-contacted cells. Preferably, the term includes a retardation of cell proliferation that is at least 50% of non-contacted cells. More preferably, the term includes a retardation of cell proliferation that is 100% of non-contacted cells (*i.e.*, the contacted cells do not increase in number or size). Most preferably, the term includes a reduction in the number or size of contacted cells, as compared to non-contacted cells. Thus, HDAC-7 or HDAC-8 antisense oligonucleotides or HDAC-7 and/or HDAC-8 small molecule inhibitors that inhibit cell proliferation in a contacted cell may induce the

contacted cell to undergo growth retardation, growth arrest, programmed cell death (*i.e.*, to apoptose), or necrotic cell death.

The anti-neoplastic utility of the antisense oligonucleotides according to the invention is described in detail elsewhere in this specification.

In yet other preferred embodiments, the cell contacted with HDAC-7 or HDAC-8 antisense oligonucleotide is also contacted with HDAC-7 and/or HDAC-8 small molecule inhibitor.

As used herein, the term "histone deacetylase small molecule inhibitor" denotes an active moiety capable of interacting with one or more specific histone deacetylase isoforms at the protein level and reducing the activity of that histone deacetylase isoform. Particularly preferred are histone deacteylase small molecule inhibitors that inhibit the HDAC-7 and/or the HDAC-8 isoform. An HDAC-1 small molecule inhibitor is a molecule that reduces the activity of the HDAC-1 isoform. An HDAC-7 small molecule inhibitor is a molecule that reduces the activity of the HDAC-7 isoform. An HDAC-8 small molecule inhibitor is a molecule that reduces the activity of the HDAC-8 isoform . In a preferred embodiment, the reduction of activity is at least 5-fold, more preferably at least 10-fold, most preferably at least 50-fold. In another embodiment, the activity of the histone deacetylase isoform is reduced 100-fold. As discussed below, a preferred histone deacetylase small molecule inhibitor is one that interacts with and reduces the enzymatic activity of HDAC-7 and/or the HDAC-8 isoform that is involved in tumorigenesis.

In a few preferred embodiments, the histone deacetylase small molecule inhibitor is operably associated with the antisense oligonucleotide. As mentioned above, the antisense oligonucleotides according to the invention may optionally be formulated well known pharmaceutically acceptable carriers or diluents. This formulation may further contain one or more one or more additional histone deacetylase antisense

oligonucleotide(s), and/or one or more histone deacetylase small molecule inhibitor(s), or it may contain any other pharmacologically active agent.

The term "operably associated with" or "operable association" includes any association between the antisense oligonucleotide and the histone deacetylase small molecule inhibitor which allows an antisense oligonucleotide to inhibit one or more specific histone deacetylase isoform-encoding nucleic acid expression and allows the histone deacetylase small molecule inhibitor to inhibit specific histone deacetylase isoform enzymatic activity. One or more antisense oligonucleotide of the invention may be operably associated with one or more histone deacetylase small molecule inhibitor. In some preferred embodiments, an antisense oligonucleotide of the invention that targets one particular histone deacetylase isoform (*e.g.*, HDAC-7 or HDAC-8) is operably associated with an small molecule inhibitor which targets the same histone deacetylase isoform. A preferred operable association is a hydrolyzable. Preferably, the hydrolyzable association is a covalent linkage between the antisense oligonucleotide and the histone deacetylase small molecule inhibitor. Such a covalent linkage is hydrolyzable, for example, by esterases and/or amidases. Examples of such hydrolyzable associations are well known in the art. Phosphate esters are particularly preferred.

In certain preferred embodiments, the covalent linkage may be directly between the antisense oligonucleotide and the histone deacetylase small molecule inhibitor so as to integrate the histone deacetylase small molecule inhibitor into the backbone of the oligonucleotide. Alternatively, the covalent linkage may be through an extended structure and may be formed by covalently linking the antisense oligonucleotide to the histone deacetylase small molecule inhibitor through coupling of both the antisense oligonucleotide and the histone deacetylase small molecule inhibitor to a carrier molecule such as a carbohydrate, a peptide, a lipid or a glycolipid. Another useful operable associations include lipophilic association, such as the formation of a liposome

containing an antisense oligonucleotide and the histone deacetylase small molecule inhibitor covalently linked to a lipophilic molecule. Such lipophilic molecules include, without limitation, phosphotidylcholine, cholesterol, phosphatidylethanolamine, and synthetic neoglycolipids, such as syalyllacNAc-HDPE. In certain preferred embodiments, the operable association may not be a physical association, but simply a simultaneous co-existence in the body, for example, when the antisense oligonucleotide is associated with one liposome and the small molecule inhibitor is associated with another liposome.

In a third aspect, the invention provides a method for inhibiting neoplastic cell proliferation in an animal, comprising administering to an animal having at least one neoplastic cell present in its body a therapeutically effective amount of a specific inhibitor of HDAC-7 and/or 8, whereby neoplastic cell proliferation is inhibited in the animal. In an embodiment thereof, the invention provides a method for inhibiting neoplastic cell growth in an animal. In this method, a therapeutically effective amount of the antisense oligonucleotide of the invention is administered to an animal having at least one neoplastic cell present in its body, the oligonucleotide being administered with a pharmaceutically acceptable carrier for a therapeutically effective period of time. Preferably, the animal is a mammal, particularly a domesticated mammal. Most preferably, the animal is a human.

The term "neoplastic cell" is used to denote a cell that shows aberrant cell growth. A neoplastic cell may be a hyperplastic cell, a cell that shows a lack of contact inhibition of growth *in vitro*, a benign tumor cell that is incapable of metastasis *in vivo*, or a cancer cell that is capable of metastases *in vivo* and that may recur after attempted removal. The term "tumorigenesis" is used to denote the induction of uncharacteristic or untimely cell proliferation that leads to the development of a neoplastic growth.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, *i.e.*, inhibiting HDAC activity, particularly HDAC-7 and/or HDAC-8 activity or to inhibit neoplastic growth or for the treatment of proliferative diseases and disorders. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

Administration of the synthetic oligonucleotide of the invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as intraocular, oral ingestion, inhalation, or cutaneous, subcutaneous, intramuscular, or intravenous injection.

When a therapeutically effective amount of synthetic oligonucleotide of the invention is administered orally, the synthetic oligonucleotide will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% synthetic oligonucleotide and preferably from about 25 to 90% synthetic oligonucleotide. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of the synthetic oligonucleotide and preferably from about 1 to 50% synthetic oligonucleotide.

When a therapeutically effective amount of synthetic oligonucleotide of the invention is administered by intravenous, subcutaneous, intramuscular, intraocular, or intraperitoneal injection, the synthetic oligonucleotide will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, subcutaneous, intramuscular, intraperitoneal, or intraocular injection should contain, in addition to the synthetic oligonucleotide, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of synthetic oligonucleotide in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patent has undergone. Ultimately, the attending physician will decide the amount of synthetic oligonucleotide with which to treat each individual patient. Initially, the attending physician will administer low doses of the synthetic oligonucleotide and observe the patient's response. Larger doses of synthetic oligonucleotide may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 10 μg to about 20 mg of synthetic oligonucleotide per kg body or organ weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. Ultimately the

attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

In a preferred embodiment, the therapeutic composition of the invention is administered systemically at a sufficient dosage to attain a blood level of antisense oligonucleotide from about 0.01 μM to about 20 μM. In a particularly preferred embodiment, the therapeutic composition is administered at a sufficient dosage to attain a blood level of antisense oligonucleotide from about 0.05 μM to about 15 μM. In a more preferred embodiment, the blood level of antisense oligonucleotide is from about 0.1 μM to about 10 μM.

For localized administration, much lower concentrations than this may be therapeutically effective. Preferably, a total dosage of antisense oligonucleotide will range from about 0.1 mg to about 200 mg oligonucleotide per kg body weight per day. In a more preferred embodiment, a total dosage of antisense oligonucleotide will range from about 1 mg to about 20 mg oligonucleotide per kg body weight per day. In a most preferred embodiment, a total dosage of antisense oligonucleotide will range from about 1 mg to about 10 mg oligonucleotide per kg body weight per day. In a particularly preferred embodiment, the therapeutically effective amount of HDAC-7 or HDAC-8 antisense oligonucleotide is about 5 mg oligonucleotide per kg body weight per day.

The method may further comprise administering to the animal a therapeutically effective amount of an HDAC-7 and/or HDAC-8 small molecule inhibitor with a pharmaceutically acceptable carrier for a therapeutically effective period of time. In some preferred embodiments, the histone deacetylase small molecule inhibitor is operably associated with the antisense oligonucleotide, as described *supra*.

The histone deacetylase small molecule inhibitor-containing therapeutic composition of the invention is administered systemically at a sufficient dosage to attain a

blood level histone deacetylase small molecule inhibitor from about 0.01 μM to about 10 μM. In a particularly preferred embodiment, the therapeutic composition is administered at a sufficient dosage to attain a blood level of histone deacetylase small molecule inhibitor from about 0.05 μM to about 10 μM. In a more preferred embodiment, the blood level of histone deacetylase small molecule inhibitor is from about 0.1 μM to about 5 μM. For localized administration, much lower concentrations than this may be effective. Preferably, a total dosage of histone deacetylase small molecule inhibitor will range from about 0.01 mg to about 100 mg protein effector per kg body weight per day. In a more preferred embodiment, a total dosage of histone deacetylase small molecule inhibitor will range from about 0.1 mg to about 50 mg protein effector per kg body weight per day. In a most preferred embodiment, a total dosage of histone deacetylase small molecule inhibitor will range from about 0.1 mg to about 25 mg protein effector per kg body weight per day. In a particularly preferred embodiment, the therapeutically effective synergistic amount of histone deacetylase small molecule inhibitor (when administered with an antisense oligonucleotide) is about 5 mg per kg body weight per day.

When the method of the invention results in an improved inhibitory effect, the therapeutically effective concentrations of either or both of the nucleic acid level inhibitor (*i.e.*, antisense oligonucleotide) and the protein level inhibitor (*i.e.*,histone deacetylase small molecule inhibitor) required to obtain a given inhibitory effect are reduced as compared to those necessary when either is used individually.

Furthermore, one of skill will appreciate that the therapeutically effective synergistic amount of either the antisense oligonucleotide or the histone deacetylase inhibitor may be lowered or increased by fine tuning and altering the amount of the other component. The invention therefore provides a method to tailor the administration/treatment to the particular exigencies specific to a given animal species or

particular patient. Therapeutically effective ranges may be easily determined for example empirically by starting at relatively low amounts and by step-wise increments with concurrent evaluation of inhibition.

In a fourth aspect, the invention provides a method for inhibiting the HDAC-7 and/or HDAC-8 isoform in a cell comprising contacting the cell with a small molecule inhibitor of the first aspect of the invention. In certain preferred embodiments of the fourth aspect of the invention, cell proliferation is inhibited in the contacted cell. In preferred embodiments, the cell is a neoplastic cell which may be in an animal, including a human, and which may be in a neoplastic growth.

In a fifth aspect, the invention provides a method for inhibiting neoplastic cell growth in an animal comprising administering to an animal having at least one neoplastic cell present in its body a therapeutically effective amount of a small molecule inhibitor of the first aspect of the invention with a pharmaceutically acceptable carrier for a therapeutically effective period of time.

The histone deacetylase small molecule inhibitor-containing therapeutic composition of the invention is administered systemically at a sufficient dosage to attain a blood level histone deacetylase small molecule inhibitor from about 0.01 μ M to about 10 μM. In a particularly preferred embodiment, the therapeutic composition is administered at a sufficient dosage to attain a blood level of histone deacetylase small molecule inhibitor from about 0.05 μM to about 10μM. In a more preferred embodiment, the blood level of histone deacetylase small molecule inhibitor is from about 0.1μM to about 5μM. For localized administration, much lower concentrations than this may be effective. Preferably, a total dosage of histone deacetylase small molecule inhibitor ranges from about 0.01 mg to about 100 mg protein effector per kg body weight per day. In a more preferred embodiment, a total dosage of histone deacetylase small molecule inhibitor

ranges from about 0.1 mg to about 50 mg protein effector per kg body weight per day. In a most preferred embodiment, a total dosage of histone deacetylase small molecule inhibitor will range from about 0.1 mg to about 25 mg protein effector per kg body weight per day.

In a sixth aspect, the invention provides a method of inhibiting the induction of cell proliferation, comprising contacting a cell with an antisense oligonucleotide that inhibits the expression of HDAC-7 or HDAC-8 or contacting a cell with a small molecule inhibitor of HDAC-7 and/or HDAC-8. In certain preferred embodiments, the cell is a neoplastic cell, and the induction of cell proliferation is tumorigenesis.

The invention further provides for histone deacetylase small molecule inhibitors that may be generated which specifically inhibit the histone deacetylase isoform(s) required for inducing cell proliferation, *e.g.*, HDAC-7 and HDAC-8, while not inhibiting other histone deacetylase isoforms not required for inducing cell proliferation. Accordingly, in a seventh aspect, the invention provides a method for identifying a small molecule histone deacetylase inhibitor that inhibits the HDAC-7 and/or HDAC-8 isoform, which is required for the induction of cell proliferation. The method comprises contacting the HDAC-7 and/or the HDAC-8 isoform with a candidate small molecule inhibitor and measuring the enzymatic activity of the contacted histone deacetylase isoform, wherein a reduction in the enzymatic activity of the contacted histone deacetylase isoform identifies the candidate small molecule inhibitor as a small molecule histone deacetylase inhibitor that inhibits the histone deacetylase isoform, *i.e.*, HDAC-7 and/or HDAC-8.

Measurement of the enzymatic activity of HDAC-7 or HDAC-8 may be achieved using known methodologies. For example, Yoshida *et al.* (*J. Biol. Chem.* **265**:17174-17179, 1990) describe the assessment of histone deacetylase enzymatic activity by the

detection of acetylated histones in trichostatin A treated cells. Taunton *et al.* (*Science* **272**:408-411, 1996) similarly describes methods to measure histone deacetylase enzymatic activity using endogenous and recombinant HDAC. Both Yoshida *et al.* (*J. Biol. Chem.* **265**:17174-17179, 1990) and Taunton *et al.* (*Science* **272**:408-411, 1996) are hereby incorporated by reference.

Preferably, the histone deacetylase small molecule inhibitor that inhibits the HDAC-7 and/or the HDAC-8 isoform required for induction of cell proliferation is an HDAC-7 and/or HDAC-8 small molecule inhibitor that interacts with and reduces the enzymatic activity of the HDAC-7 and/or the HDAC-8 isoform.

In an eighth aspect, the invention provides a method for identifying a small molecule histone deacetylase inhibitor that inhibits the HDAC-7 and/or HDAC-8 isoform involved in the induction of cell proliferation. The method comprises contacting a cell with a candidate small molecule inhibitor and measuring the enzymatic activity of the contacted histone deacetylase isoform, wherein a reduction in the enzymatic activity of the HDAC-7 and/or HDAC-8 isoform identifies the candidate small molecule inhibitor as a small molecule histone deacetylase inhibitor that inhibits HDAC-7 and/or HDAC-8.

In a ninth aspect, the invention provides a small molecule histone deacetylase inhibitor identified by the method of the seventh or the eighth aspects of the invention. Preferably, the histone deacetylase small molecule inhibitor is substantially pure.

In a tenth aspect, the invention provides a method for inhibiting cell proliferation in a cell comprising contacting a cell with at least two reagents selected from the group consisting of an antisense oligonucleotide that inhibits expression of HDAC-7 or HDAC-8 isoform, a small molecule histone deacetylase inhibitor that inhibits expression or activity of HDAC-7 and/or HDAC-8 isoform, an antisense oligonucleotide that inhibits expression of the HDAC-1 isoform, a small molecule histone deacetylase inhibitor that

inhibits the expression or the activity of the HDAC-1 isoform, an antisense oligonucleotide that inhibits expression of a DNA methyltransferase, and a small molecule DNA methyltransferase inhibitor. In one embodiment, the inhibition of cell growth of the contacted cell is greater than the inhibition of cell growth of a cell contacted with only one of the reagents. In certain embodiments, each of the reagents selected from the group is substantially pure. In preferred embodiments, the cell is a neoplastic cell. In yet additional preferred embodiments, the reagents selected from the group are operably associated.

In an eleventh aspect, the invention provides a method of inhibiting neoplastic cell growth comprising contacting a cell with at least two reagents selected from the group consisting of an antisense oligonucleotide that inhibits expression of HDAC-7 or HDAC-8 isoform, a small molecule histone deacetylase inhibitor that inhibits the expression or the activity of HDAC-7 and/or HDAC-8 isoform, an antisense oligonucleotide that inhibits expression of the HDAC-1 isoform, a small molecule histone deacetylase inhibitor that inhibits expression or activity of the HDAC-1 isoform, an antisense oligonucleotide that inhibits expression of a DNA methyltransferase, and a small molecule DNA methyltransferase inhibitor. In one embodiment, the inhibition of cell growth of the contacted cell is greater than the inhibition of cell growth of a cell contacted with only one of the reagents that inhibit HDAC-7 or HDAC-8. In certain embodiments, each of the reagents selected from the group is substantially pure. In preferred embodiments, the cell is a neoplastic cell. In yet additional preferred embodiments, the reagents selected from the group are operably associated.

Antisense oligonucleotides that inhibit DNA methyltransferase are described in Szyf and von Hofe, U.S. Patent No. 6,054,339. DNA methyltransferase small molecule inhibitors include, without limitation, 5-aza-2'-deoxycytidine (5-aza-dC), 5-fluoro-2'-deoxycytidine, 5-aza-cytidine (5-aza-C), or 5,6-dihydro-5-aza-cytidine.

EXAMPLES

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the appended claims.

Example 1

Expression of HDAC Isotypes in Human Clinical Samples Analyzed by Human Cancer Profiling Array

Gene expression of human HDAC1, HDAC7 and HDAC8 in human cancer and the matched normal tissues at mRNA levels was determined by using Cancer Profiling Array (Clontech, Palo Alto, California). The cDNA probes of human HDAC1, HDAC7 and HDAC8 were made by PCR reactions with ^{32}P-labelled dCTP and primers corresponding to the 3′-end of the coding sequences of each HDAC isotypes. To PCR cDNA probe for HDAC1, the primer used corresponded to the nucleotide postion #1486 to 1515 for human HDAC1 gene (accession # NM_004964), with the sequence: 5′-CAT TCA GGC CAA GTC GAC CTC CTC CTT GAC-3′. To PCR HDAC7 cDNA probe, the primer used corresponded to the nucleotide position of #2858 to #2890 of human HDAC7 gene (accession # NM_015401), with sequence 5′-ATG AAT TCC TGT GCA CCC

GGA TCA CGG CCT CCA GAG AGC GG-3'. To PCR HDAC8 cDNA probe, the primer used corresponded to the nucleotide position of #1168-#1186 of human HDAC8 sequence (accession # AF_230097), with sequence 5'-CCC TCG AGG ACC ACA TGC TTC AGA TTC-3'. Templates for PCR were purified HDAC1, HDAC7 or HDAC8 gene fragments. PCR reactions were performed using Expand™ Long Template PCR system (Roche,________________,__). Hybridization of cDNA probes for human HDAC1, HDAC7 or HDAC8 to nylon array membrane was performed as suggested by the vendor (BD Biosciences Clontech, ____________,__). After hybridization and washing, array membranes were exposed to Cyclone Phosphor-Screen (Packard,________________,__)) for data analysis. Expression levels of HDAC isotypes shown in Table 1 were normalized by that of ubiquitin. As shown in Table 1, there is significant upregulation of HDAC1 expression at the RNA level in patients with uterus, ovary and lung cancers, while significant upregulation of HDAC7 or HDAC8 expression was observed in patients with colon and rectum cancers.

Example 2

Synthesis and Identification of Antisense Oligonucleotides

Antisense (AS) and mismatch (MM) oligodeoxynucleotides (oligos) were designed to be directed against the 5'- or 3'-untranslated region (UTR) of the targeted gene. Oligos listed in Table 2 were synthesized with the phosphorothioate backbone and the 4X4 nucleotides 2'-O-methyl modification on an automated synthesizer and purified by preparative reverse-phase HPLC. All oligos used were 20 base pairs in length.

To identify antisense oligodeoxynucleotide (ODN) capable of inhibiting HDAC-7 expression in human cancer cells, eighteen phosphorothioate ODNs containing sequences complementary to the 5' or 3' UTR of the human HDAC-7 gene (GenBank Accession No. AF239243) were initially screened in A549 cells at 100 nM. Cells were harvested after 24 hours of treatment, and HDAC-7 RNA expression was analyzed by Northern blot analysis. From the screen, we identified both AS-1 and AS-2 against human HDAC7 (see Table 2) with good antisense activities. Total RNAs were harvested and were analyzed by Northern Blot. GAPDH expression was analyzed to indicate total RNA loading in each lane.

To identify antisense oligodeoxynucleotides (ODN) capable of inhibiting HDAC-8 expression in human cancer cells, fourteen phosphorothioate ODNs containing sequences complementary to the 5' or 3' UTR of the human HDAC-7 gene (GenBank Accession No. AF230097) were initially screened in A549 cells at 100 nM. Cells were harvested after 24 hours of treatment, and HDAC-8 RNA expression was analyzed by Northern blot analysis. From the screen, we identified both AS-1 and AS-2 against human HDAC8 (see Table 2) with good antisense activities. Total RNAs were harvested and were analyzed by Northern Blot. GAPDH expression was analyzed to indicate total RNA loading in each lane.

Twenty-four phosphorothioate ODNs containing sequences complementary to the 5' or 3' UTR of the human HDAC-2 gene (GenBank Accession No. U31814) were screened as above. HDAC-2 AS was identified as an ODN with antisense activity to human HDAC-2. HDAC-2 MM was created as a control; compared to the antisense oligo, it contains a 7-base mismatch.

Twenty-one phosphorothioate ODNs containing sequences complementary to the 5' or 3' UTR of the human HDAC-3 gene (GenBank Accession No. AF039703) were screened as above. HDAC-3 AS was identified as an ODN with antisense activity to human HDAC-3. HDAC-3 MM oligo was created as a control; compared to the antisense oligo, it contains a a 6-base mismatch.

Seventeen phosphorothioate ODNs containing sequences complementary to the 5' or 3' UTR of the human HDAC-4 gene (GenBank Accession No. AB006626) were screened as above. HDAC-4 AS was identified as an ODN with antisense activity to human HDAC-4. HDAC-4 MM oligo was created as a control; compared to the antisense oligo, it contains a 6-base mismatch.

Thirteen phosphorothloate ODNs containing sequences complementary to the 5' or 3' untranslated regions of the human HDAC-6 gene (GenBank Accession No. AJ011972) were screened as above. HDAC-6 AS was identified as an ODN with antisense activity to human HDAC-6. HDAC-6 MM oligo was created as a control; compared to the antisense oligo, it contains a 7-base mismatch.

Example 3

HDAC AS ODNs Specifically Inhibit Expression at the mRNA Level

In order to determine the dose response of HDAC7 antisense inhibitors to reduce HDAC7 message at the mRNA level, Human A549 cells were treated with 25 or 50 nM of antisense (AS1 and AS2) oligos directed against human HDAC-7 or the corresponding mismatch of AS1 (MM1) oligo or an universal mismatch (UMM) for 24 hours. Shown in Fig. 1, both AS1 or AS2 can inhibit human HDAC7 expression at the mRNA level. The

time dependence of HDAC7 antisense inhibitors on blocking HDAC7 gene expression at the mRNA level was analyzed by treating A549 cells with 50 nM AS1 or MM1 oligos. Shown in Fig. 3, AS1 oligo can significantly block gene expression of human HDAC7 at the mRNA level by 24 hours. Similarly, A549 cells were treated with 25 nM or 50 nM of AS1 or AS2 oligos directed against human HDAC-8 or its MM oligo for AS2 (MM2) for 24 hours. The dose response of these oligos on inhibiting HDAC8 expression at mRNA level was shown in Fig. 5. AS-2 oligo against human HDAC8 at 50 nM was also used to treat A549 cells for 24 or 48 hours. Shown in Fig. 6, AS-2 oligo significantly block HDAC8 expression at the mRNA level by 24 hours.

For all ex vivo oligo treatment, human A549 human bladder carcinoma cells were seeded in 10 cm tissue culture dishes one day prior to oligonucleotide treatment. The cell lines were obtained from the American Type Culture Collection (ATCC) (Manassas, VA) and were grown under the recommended culture conditions. Before the addition of the oligonucleotides, cells were washed with PBS (phosphate buffered saline). Next, lipofectin transfection reagent (GIBCO BRL Mississauga, Ontario, CA), at a concentration of 6.25 μg/ml, was added to serum free OPTIMEM medium (GIBCO BRL, Rockville, MD), which was then added to the cells. The oligonucleotides were added directly to the cells (*i.e.*, one oligonucleotide per plate of cells). Mismatched oligonucleotides were used as controls. The same concentration of oligonucleotide (*e.g.*, 50 nM) was used per plate of cells for each oligonucleotide tested.

Cells were harvested, and total RNAs were analyzed by Northern blot analysis. Briefly, total RNA was extracted using RNeasy miniprep columns (QIAGEN). Ten to twenty μg of total RNA was run on a formaldehyde-containing 1% agarose gel with 0.5 M sodium phosphate (pH 7.0) as the buffer system. RNAs

were then transferred to nitrocellulose membranes and hybridized with the indicated radiolabeled DNA probes. Autoradiography was performed using conventional procedures.

Our results indicate that HDAC AS ODNs can specifically inhibit targeted HDAC expression at the mRNA level.

Example 4

HDAC OSDNs Inhibit HDAC Protein Expression

In order to determine whether treatment with HDAC ODNs would inhibit HDAC protein expression, human A549 cancer cells were treated with *25 or 50 nM* of paired antisense or its mismatch oligos directed against human HDAC-7 for 48 hours. ODN treatment conditions were as previously described. *To analyze the time course of AS oligos on inhibition of HDAC7 protein expression, A549 cells were treated with oligos (AS1, AS2 or UMM, each 50 nM) for either 24 hours or 48 hours.*

Cells were lysed in buffer containing 1% Triton X- 100, 0.5 % sodium deoxycholate, 5 mM EDTA, 25 mM Tris-HC1, pH 7.5, plus protease inhibitors. Total protein was quantified by the protein assay reagent from Bio-Rad (Hercules, CA). 100 ug of total protein was analyzed by SDS-PAGE. Next, total protein was transferred onto a PVDF membrane and probed with HDAC-7-specific primary antibodies. As shown in Figure 2, the treatment of cells with HDAC-7 ODNs for 48 hours specifically inhibits the expression of HDAC-7 isotype protein.

Results from Fig. 2 and Fig. 4 clearly demonstrate that HDAC7 AS oligos can inhibit expression of human HDAC7 at the protein level.

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(In order to demonstrate that the level of HDAC protein expression is an important factor in the cancer cell phenotype, experiments were done to determine the level of HDAC isotype expression in normal and cancer cells. Western blot analysis was performed as described above.

The results are presented in Table 1 and clearly demonstrate that HDAC isotype proteins are aberrantly expressed in cancer cell lines.)

Example 5

Effect of HDAC Isotype Specific OSDNs on Cell Growth and Apoptosis

In order to determine the effect of HDAC ODNs on cell growth inhibition and cell death through apoptosis, A549, T24, Du145, HCT116 cells (ATCC, Manassas, VA), or HMEC cells (BioWhittaker, Walkersville, Maryland) were treated with HDAC ODNs as previously described.

For the apoptosis study, cells were analyzed using the Cell Death Detection ELISA ^{Plus} kit (Roche Diagnostic GmBH, Mannheim, Germany) according to the manufacturer's directions. Typically, 10,000 cells were plated in 96-well tissue culture dishes for 2 hours before harvest and lysis. Each sample was analyzed in duplicate. ELISA reading was done using a MR700 plate reader (DYNEX Technology, Ashford, Middlesex, England) at 410 nm. The reference

was set at 490 nm.

For the cell growth *inhibition* analysis, human cancer or normal cells were treated with 50 nM of paired AS or MM oligos directed against human *HDAC-1, HDAC-7 or HDAC-8 for up to 96 hours. Cell numbers were counted every day by trypan blue exclusion. Percentage of inhibition was calculated as (100 - AS cell numbers/control cell numbers)%.*

Results of the study are shown in Figures 7, 8, 11-16, and in Table 3. Treatment of human cancer cells by HDAC1, HDAC-7 AS, and HDAC 8 AS induces growth arrest of various human cancer cells. Treatment of human cancer cells by HDAC1 or HDAC-8 AS induces growth arrest of various human cancer cells but not normal cells. The corresponding mismatches have no effect. Since T24 cells are p53 null and A549 cells are p53 wild type, this induction of apoptosis is independent of p53 activity.

Example 6

Synergy of Isotype-Specific Antisense Inhibitors Directed Against Human HDAC7 or HDAC8 with Antisense Inhibitor Directed Against Human HDAC1 on Induction of Apopotosis of Human Cancer Cells

Human A549 cells were treated with each isotype-specific AS inhibitors against human HDAC1-8 at 40 nM alone, or with 20 nM of HDAC AS oligos in addition to 20 nM of UMM control oligo, or with 40 nM of UMM control oligo .

Similarly, A549 cells were treated with 20 nM of HDAC1 AS in combination with 20 nM of each of AS inhibitors against human HDAC2 to HDAC8. After 48 hour treatment, A549 cells were harvested and analyzed for apoptosis by ELISA as described previously. Apoptosis of A549 cells by AS inhibition was compared to that of cells treated with 1 uM TSA for 16 hours. Shown in Fig. 16, HDAC7 and HDAC8 AS inhibitors can synergize with HDAC1 AS inhibitor to induce significant apoptosis of human cancer A549 cells, while AS inhibitors against other HDAC isotypes did not synergize with HDAC1 AS. The control oligo UMM had no effect on induction of apoptosis. Specific inhibition of HDAC7 with HDAC1 or inhibition of HDAC8 with HDAC1 by their AS inhibitors resulted in even more dramatic induction of apoptosis in A549 cells than that by TSA treatment.

Example 7:

Effect of HDAC Isotype-Specific Antisense Inhibitors on Cell Cycle Blocks of Human Cancer Cells.

Human cancer cells (typically A549 cells) were treated with HDAC isotype-specific antisense ODNs or their mismatch or control ODNs for 48 hours. Cells were harvested and fixed by 70% ethanol at -20°C. Nucleic acids from fixed cells were stained with propidium iodide (50 μg/ml). Cell cycle profiles of treated cancer cells were measured by using a fluorescence-activated cell sorter (FACScan, from Becton Dickson).

Shown in Fig. 9 and 10, antisense inhibitors of human HDAC7 or HDAC8 clearly induced cell cycle blocks of human cancerA549 cells at G2/M phase.

Example 8:

Effect of HDAC Isotype-Specific Small Molecule Inhibitors on Growth Inhibition of Various Human Cancer Cells in vitro

Effects of HDAC isotype-specific small molecule inhibitors on growth inhibition of various human cancer cells (from ATCC) in vitro were determined by MTT assays. Briefly, cells seeded in 96-well plates were incubated for 72 hours at 37°C in 5% CO_2 incubator. MTT (Sigma) was added at a final concentration of 0.5 mg/ml and incubated with the cells for 4 hours before an equal volume of solubilization buffer (50% N,N-dimethylformamide, 20% SDS, pH 4.7) was added onto cultured cells. After overnight incubation, solubilized dye was quantified by colorimetric reading at 570 nM using a reference at 630 nM. OD values were converted to cell numbers according to a standard growth curve of the relevant cell line. The concentration which reduces cell numbers to 50% of those of DMSO-treated cells is determined as MTT IC_{50}. In Table 4, IC50s of several HDAC7 or HDAC8 inhibitors in MTT assays in various human cancer cell lines were listed. They include colon cancer cells HCT116 and SW48, lung cancer cells A549 and H446, breast cancer cells MCF-7 and MDAmb231, a prostate cancer cell line Du145 and a bladder cancer cell line T24. As shown in Table4, all molecules can inhibit growth of human cancer cells in vitro.

Example 9

Inhibition by Small Molecules of Tumor Growth in a Mouse Model

Female BALB/c nude mice are obtained from Charles River Laboratories (Charles River, NY) and used at age 8-10 weeks. Human tumor cells *(2 x 10^6, colon carcinoma cells HCT116 or SW48, lung carcinoma cells A549, pancreatic carcinoma Panc-1, ovarian carcinoma cells ES2, or prostate carcinoma cells Du145)* are injected subcutaneously in the animal's flank and allowed to form solid tumors. Tumor fragments are serially passaged a minimum of three times, then approximately 30 mg tumor fragments are implanted subcutaneously through a small surgical incision under general anaesthesia. Small molecule inhibitor administration by intraperotineal or oral administration was initiated when the tumors reached a volume of 100 mm^3. For intraperotineal administration, small molecule inhibitors of HDAC-7, HDAC- 8 or both, or in combination with small molecule inhibitors of HDAC-1 *(20–60 mg/kg body weight/day)* are dissolved in 100% DMSO and administered daily by injection. For oral administration, small molecule inhibitors of HDAC *(60 mg/kg body weight)* are dissolved in *saline acidified with 0.2 N HCl*. Tumor volumes are monitored twice weekly up to 20 days. Each experimental group contains at least 6-8 animals. Percentage inhibition is calculated using volume of tumor from vehicle-treated mice as controls. *Shown in Table 4, inhibition of HDAC7 or HDAC8 in combination with HDAC1 leads to inhibition of growth of various human tumors in vivo.*

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodi,emts of the invention described herein. Such equivalents are intended to be encompasssed by the following claims.

What is claimed is:

1. A method of inhibiting HDAC-7 activity in a cell, comprising contacting the cell with an antisense oligonucleotide complementary to a region of RNA that encodes a portion of HDAC-7, whereby HDAC-7 activity is inhibited.

2. The method according to claim 1, wherein the cell is contacted with an HDAC-7 antisense oligonucleotide that is a chimeric oligonucleotide.

3. The method according to claim 1, wherein the cell is contacted with an HDAC-7 antisense oligonucleotide that is a hybrid oligonucleotide.

4. The method according to claim 1, wherein the antisense oligonucleotide has a nucleotide sequence of from about 13 to about 35 nucleotides which is selected from the nucleotide sequence of SEQ ID NO:1.

5. The method according to claim 1, wherein the antisense oligonucleotide has a nucleotide sequence of from about 15 to about 26 nucleotides which is selected from the nucleotide sequence of SEQ ID NO:1.

6. The method according to claim 1, wherein the cell is contacted with an HDAC-8 antisense oligonucleotide that is SEQ ID NO: 3.

7. The method according to claim 1, whereby inhibition of HDAC-7 activity in the contacted cell further leads to an inhibition of cell proliferation in the contacted cell.

8. The method according to claim 1, wherein inhibition of HDAC-7 activity in the contacted cell further leads to growth retardation of the contacted cell.

9. The method according to claim 1, wherein inhibition of HDAC-7 activity in the contacted cell further leads to growth arrest of the contacted cell.

10. The method according to claim 1, wherein inhibition of HDAC-7 activity in the contacted cell further leads to programmed cell death of the contacted cell.

11. The method according to claim 8, wherein inhibition of HDAC-7 activity in the contacted cell further leads to necrotic cell death of the contacted cell.

12. A method of inhibiting HDAC-7 or HDAC-8 activity in a cell, comprising contacting the cell with a small molecule inhibitor of HDAC-7 selected from the group consisting of: N-(2-Aminophenyl)-3-{4-[(3,4,5-trimethoxyphenylamino)-methyl]-phenyl} acrylamide, N-(2-Amino-phenyl)-4-{[6-(2-morpholin-4-yl-ethoxy)-benzothiazol-2-ylamino]-methyl}-benzamide, N-(2-Aminophenyl)-4-[(6-methoxypyridin-3-ylamino)-methyl]-benzamide, N-(2-Aminophenyl)-4-[(6-methoxypyridin-3-ylamino)-methyl]-benzamide, N-(2-Aminophenyl)-4-benzo[1,3]-dioxol-5-ylaminomethyl)-benzamide, N-(2-Aminophenyl)-4-(5-methoxy-1H-benzimidazol-2-yl-sulfanylmethyl)-benzamide, N-(2-Amino-phenyl)-4-[(4-morpholin-4-yl-phenylamino)-methyl]-benzamide, N-(2-Amino-phenyl)-4-{[4-(3,4-dimethoxy-phenyl)-pyrimidin-2-ylamino]-methyl}-benzamide, *N*-(2-Aminophenyl)-4-[1-(3,4-dimethoxyphenyl)-3-(4-methylsulfanylphenyl)-ureidomethyl]-benzamide, and N-(2-Amino-phenyl)-4-{[2-(2-methoxy-ethyl)-1,3-dioxo-2,3-dihydro-1H-isoindol-5-ylamino]-methyl}-benzamide.

13. The method according to claim 12, whereby inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to an inhibition of cell proliferation in the contacted cell.

14. The method according to claim 12, wherein inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to growth retardation of the contacted cell.

15. The method according to claim 12, wherein inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to growth arrest of the contacted cell.

16. The method according to claim 12, wherein inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to programmed cell death of the contacted cell.

17. The method according to claim 13, wherein inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to necrotic cell death of the contacted cell.

18. A method for inhibiting neoplastic cell proliferation in an animal, comprising administering to an animal having at least one neoplastic cell present in its body a therapeutically effective amount of an antisense oligonucleotide complementary to a region of RNA that encodes a portion of HDAC-7 or HDAC-8, whereby neoplastic cell proliferation is inhibited.

19. The method according to claim 18, wherein the animal is administered a chimeric HDAC-7 or HDAC-8 antisense oligonucleotide.

20. The method according to claim 18, wherein the animal is administered a hybrid HDAC-7 or HDAC-8 antisense oligonucleotide.

21. The method according to claim 18, wherein the antisense oligonucleotide has a nucleotide sequence of from about 13 to about 35 nucleotides which is selected from the nucleotide sequence of SEQ ID NO:1.

22. The method according to claim 18, wherein the antisense oligonucleotide has a nucleotide sequence of from about 15 to about 26 nucleotides which is selected from the nucleotide sequence of SEQ ID NO:1.

23. The method according to claim 18, wherein the antisense oligonucleotide has a nucleotide sequence of from about 20 to about 26 nucleotides which is selected from the nucleotide sequence of SEQ ID NO:2.

24. The method according to claim 18, wherein the cell is contacted with an HDAC-8 antisense oligonucleotide that is SEQ ID NO: 3.

25. The method according to claim 18, whereby inhibition of HDAC-8 activity in the contacted cell further leads to an inhibition of cell proliferation in the contacted cell.

26. The method according to claim 18, wherein inhibition of HDAC-8 activity in the contacted cell further leads to growth retardation of the contacted cell.

27. The method according to claim 18, wherein inhibition of HDAC-8 activity in the contacted cell further leads to growth arrest of the contacted cell.

28. The method according to claim 18, wherein inhibition of HDAC-8 activity in the contacted cell further leads to programmed cell death of the contacted cell.

29. The method according to claim 25, wherein inhibition of HDAC-8 activity in the contacted cell further leads to necrotic cell death of the contacted cell.

30. A method for inhibiting neoplastic cell proliferation in an animal, comprising administering to an animal having at least one neoplastic cell present in its body a therapeutically effective amount of a small molecule inhibitor of HDAC-7 or HDAC-8

31. The method according to claim 30, whereby inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to an inhibition of cell proliferation in the contacted cell.

32. The method according to claim 30, wherein inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to growth retardation of the contacted cell.

33. The method according to claim 30, wherein inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to growth arrest of the contacted cell.

34. The method according to claim 30, wherein inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to programmed cell death of the contacted cell.

35. The method according to claim 31, wherein inhibition of HDAC-7 or HDAC-8 activity in the contacted cell further leads to necrotic cell death of the contacted cell.

36. The method according to claim 18 or 30, wherein the animal is a human.

37. The method according to claim 18 or 30, further comprising administering to an animal a therapeutically effective amount of an antisense oligonucleotide complementary to a region of RNA that encodes a portion of HDAC-1.

38. The method according to claim 37, wherein the animal is administered a chimeric HDAC-1 antisense oligonucleotide.

39. The method according to claim 37, wherein the animal is administered a hybrid HDAC-1 antisense oligonucleotide.

40. The method according to claim 37, wherein the animal is administered an HDAC-1 antisense oligonucleotide having a nucleotide sequence of from about 13 to about 35 nucleotides which is selected from the nucleotide sequence of SEQ ID NO: 1.

41. The method according to claim 37, wherein the animal is administered an HDAC-1 antisense oligonucleotide having a nucleotide sequence of from about 15 to about 26 nucleotides which is selected from the nucleotide sequence of SEQ ID NO: 1.

42. The method according to claim 37, wherein the animal is administered an HDAC-1 antisense oligonucleotide having a nucleotide sequence of from about 20 to about 26 nucleotides which is selected from the nucleotide sequence of SEQ ID NO: 2.

43. The method according to claim 37, wherein the animal is administered an HDAC-1 antisense oligonucleotide that is SEQ ID NO: 2.

44. The method according to claim 18 or 30, further comprising administering to an animal a therapeutically effective amount of a small molecule inhibitor of HDAC-1.

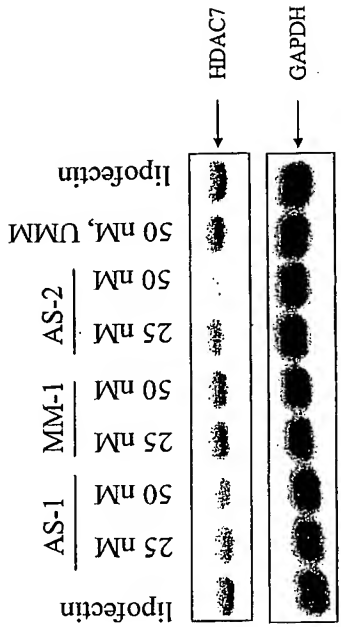

lipofectin
AS-1
25 nM
50 nM
MM-1
25 nM
50 nM
AS-2
25 nM
50 nM
50 nM, UMM
lipofectin
HDAC7
GAPDH

Fig. 1

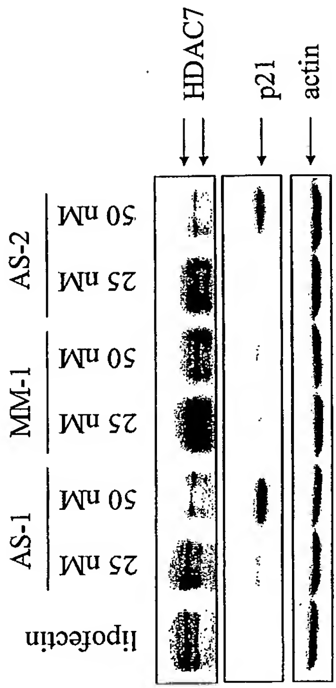
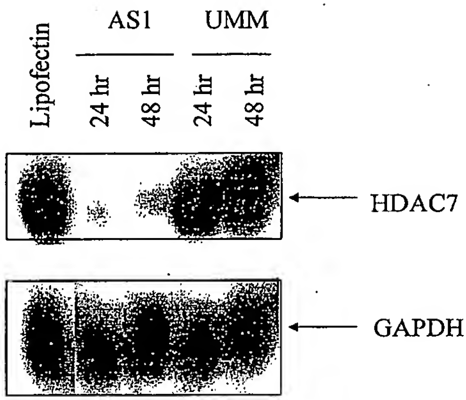

lipofectin
AS-1
25 nM
50 nM
MM-1
25 nM
50 nM
AS-2
25 nM
50 nM
HDAC7
p21
actin

Fig. 2

Fig. 3



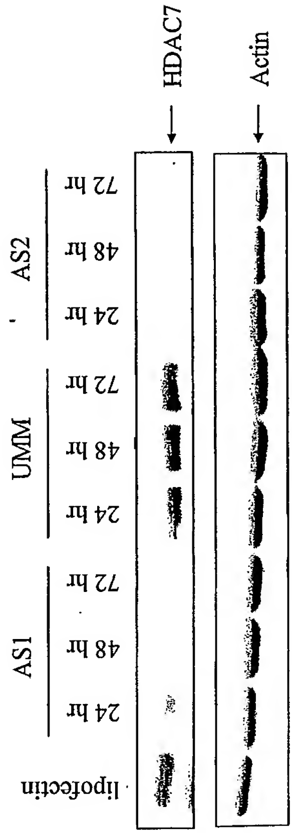

lipofectin
AS1
24 hr
48 hr
72 hr
UMM
24 hr
48 hr
72 hr
AS2
24 hr
48 hr
72 hr
HDAC7
Actin

Fig. 4

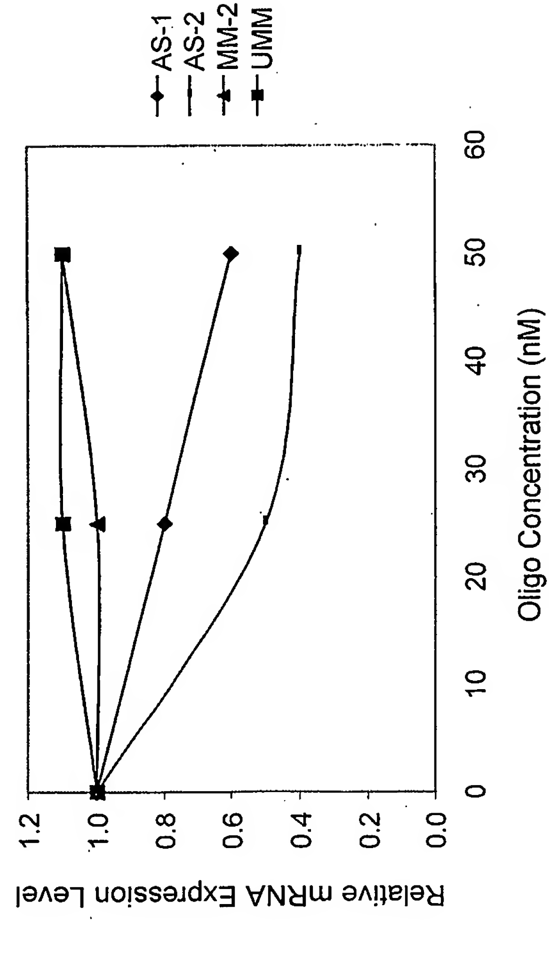

AS-1
AS-2
MM-2
UMM
Relative mRNA Expression Level
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Oligo Concentration (nM)

Fig. 5

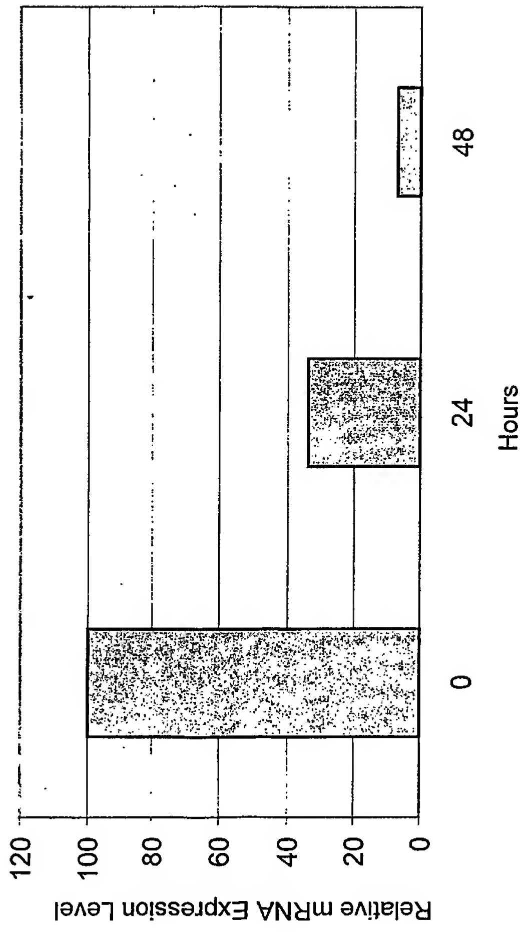

Relative mRNA Expression Level
120
100
80
60
40
20
0
0
24
48
Hours

Fig. 6

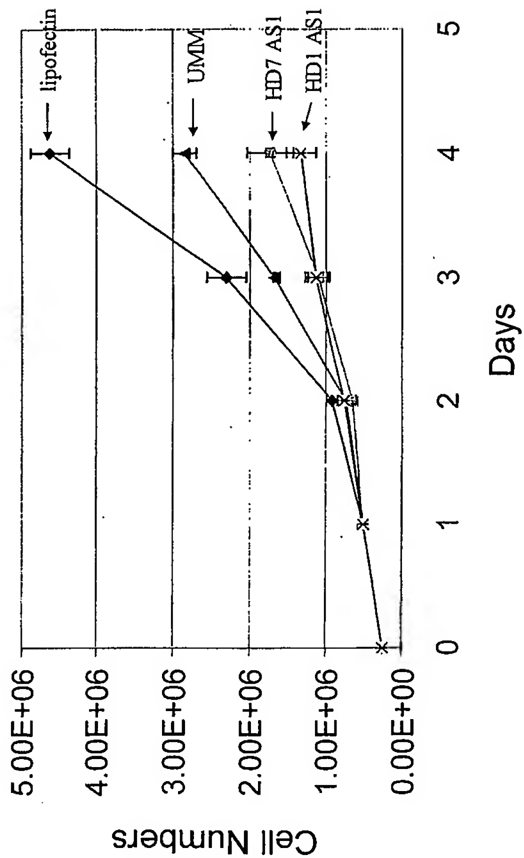
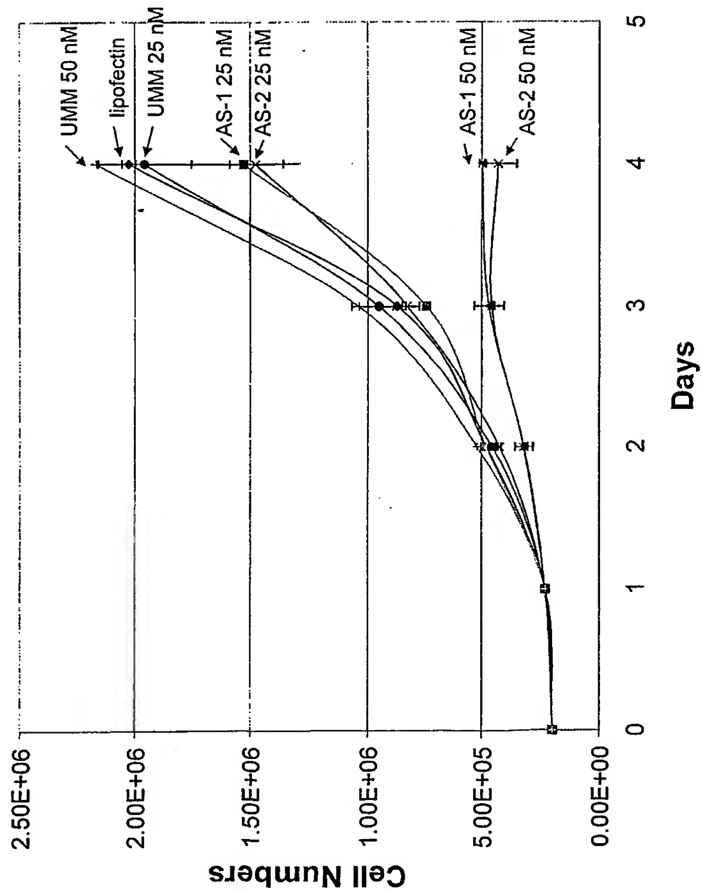

lipofectin
UMM
HD7 AS1
HD1 AS1
Cell Numbers
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+00
0
1
2
3
4
5
Days

Fig. 7

Fig. 8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1.50E+06
1.00E+06
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0.00E+00
Days
0
1
2
3
4
5
UMM 50 nM
lipofectin
UMM 25 nM
AS-1 25 nM
AS-2 25 nM
AS-1 50 nM
AS-2 50 nM

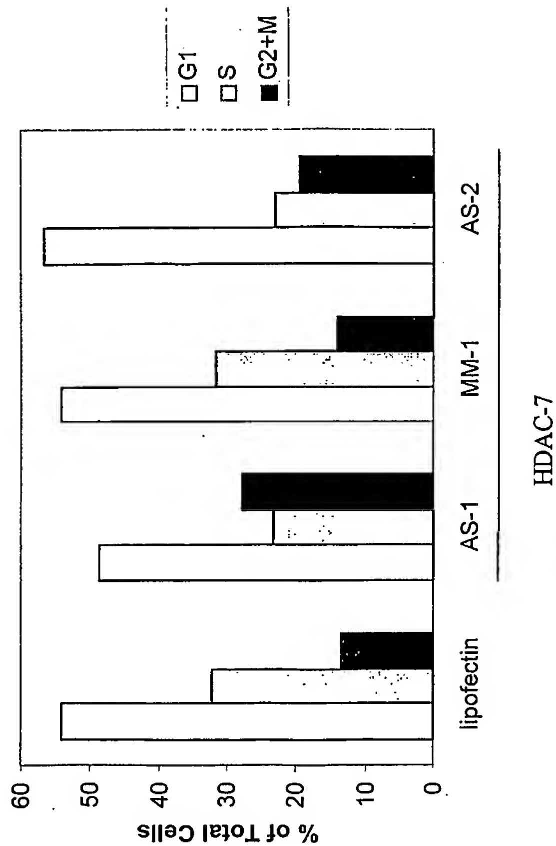
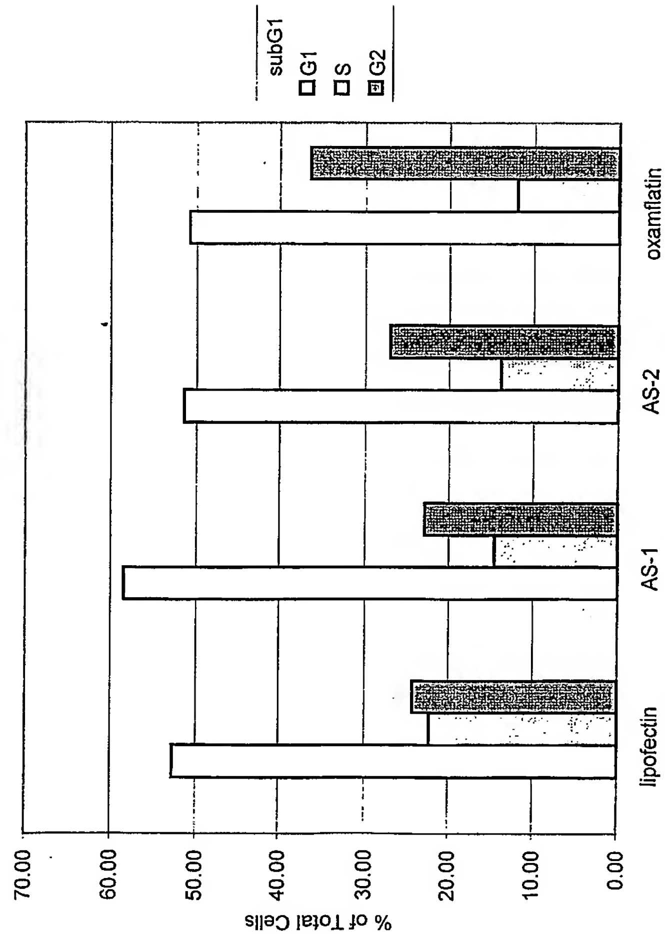

G1
S
G2+M
% of Total Cells
0
10
20
30
40
50
60
lipofectin
AS-1
MM-1
AS-2
HDAC-7

Fig. 9

Fig. 10


subG1
G1
S
G2
% of Total Cells
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60.00
50.00
40.00
30.00
20.00
10.00
0.00
lipofectin
AS-1
AS-2
oxamflatin

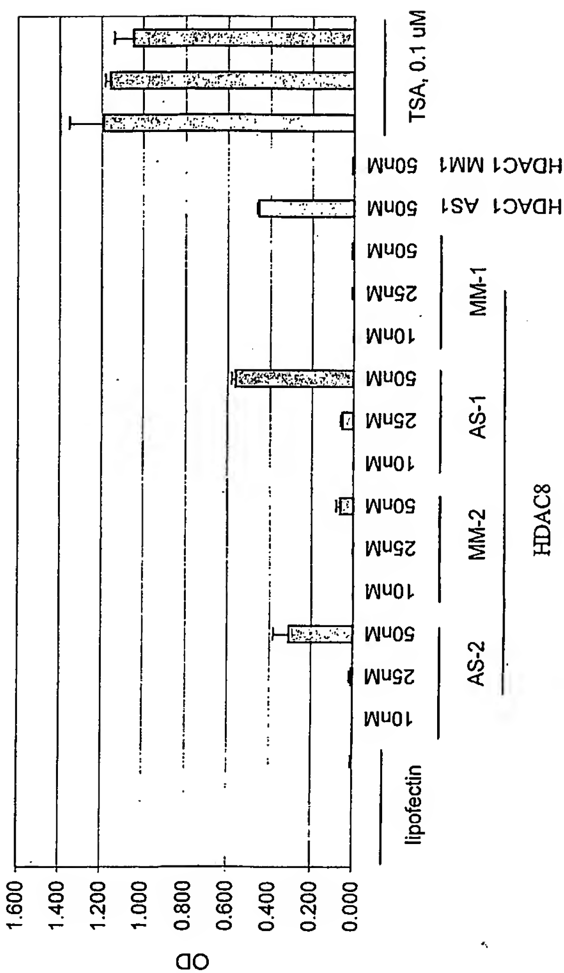

OD
0.000
0.200
0.400
0.600
0.800
1.000
1.200
1.400
1.600
lipofectin
10nM
25nM
50nM
AS-2
10nM
25nM
50nM
MM-2
10nM
25nM
50nM
AS-1
10nM
25nM
50nM
MM-1
HDAC8
50nM HDAC1 AS1
50nM HDAC1 MM1
TSA, 0.1 uM

Fig. 11

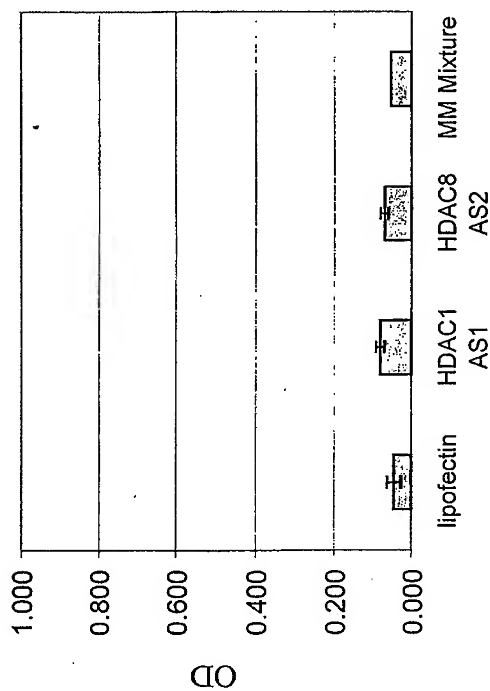
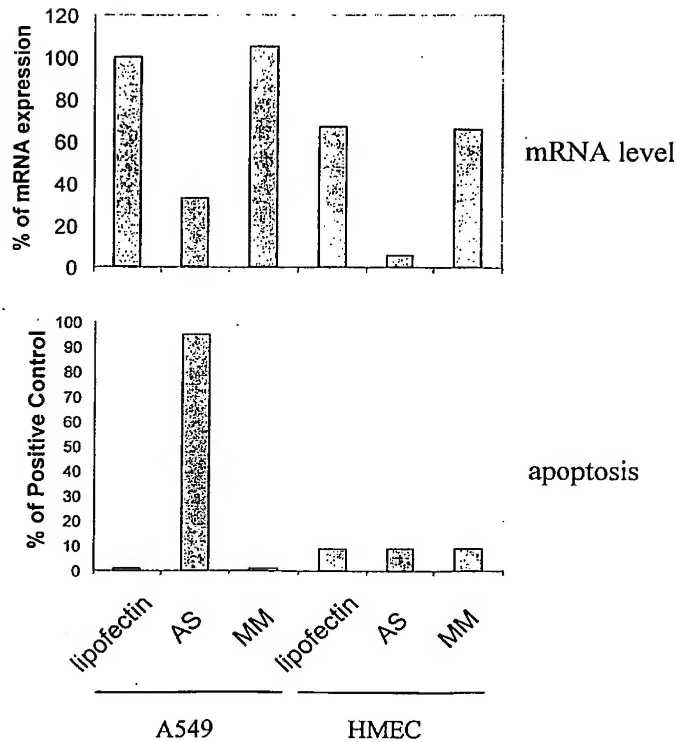

OD
1.000
0.800
0.600
0.400
0.200
0.000
lipofectin
HDAC1 AS1
HDAC8 AS2
MM Mixture

Fig. 12

Fig. 13


120
100
80
60
40
20
0
% of mRNA expression
mRNA level
100
90
80
70
60
50
40
30
20
10
0
% of Positive Control
apoptosis
lipofectin
AS
MM
lipofectin
AS
MM
A549
HMEC

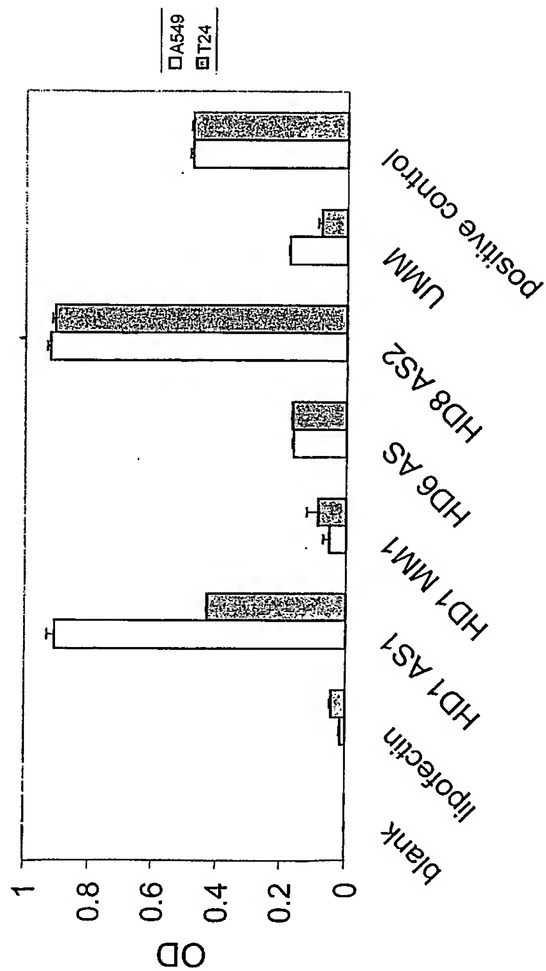

A549
T24
OD
1
0.8
0.6
0.4
0.2
0
blank
lipofectin
HD1 AS1
HD1 MM1
HD6 AS
HD8 AS2
UMM
positive control

Fig. 14

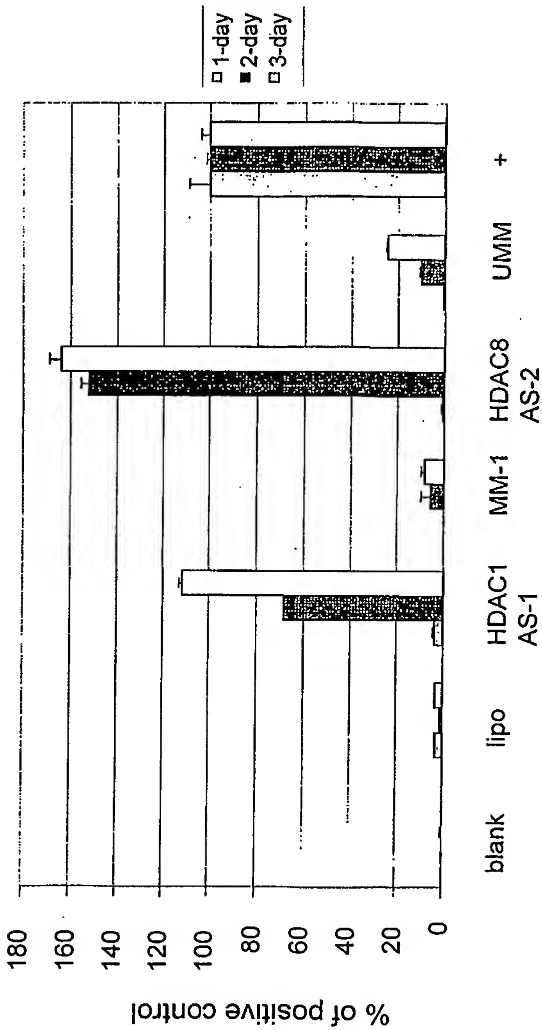

1-day
2-day
3-day
% of positive control
180
160
140
120
100
80
60
40
20
0
blank
lipo
HDAC1 AS-1
MM-1
HDAC8 AS-2
UMM
+

Fig. 15

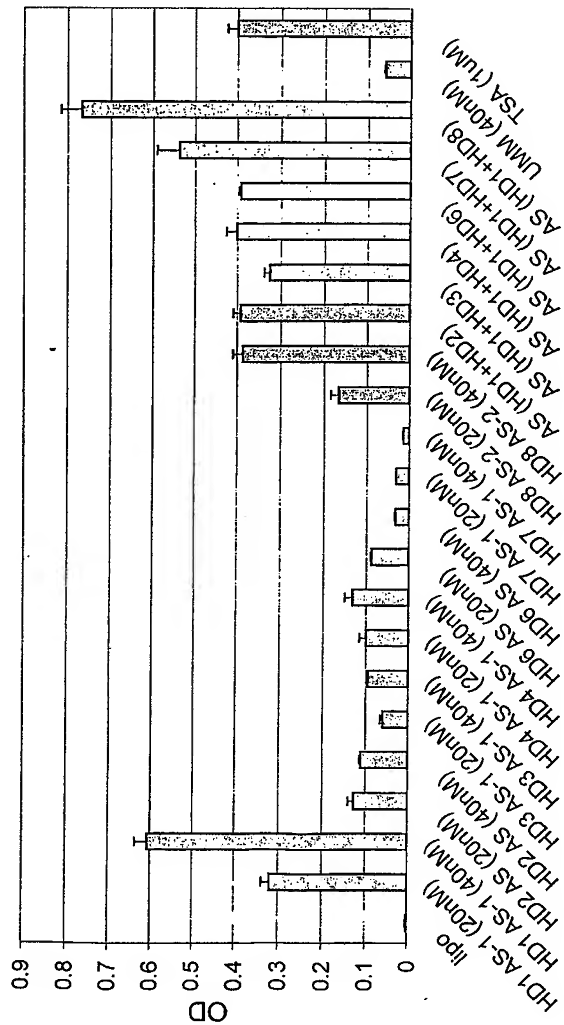

OD
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
lipo
HD1 AS-1 (20nM)
HD1 AS-1 (40nM)
HD2 AS (20nM)
HD2 AS (40nM)
HD3 AS-1 (20nM)
HD3 AS-1 (40nM)
HD4 AS-1 (20nM)
HD4 AS-1 (40nM)
HD6 AS (20nM)
HD6 AS (40nM)
HD7 AS-1 (20nM)
HD7 AS-1 (40nM)
HD8 AS-2 (20nM)
HD8 AS-2 (40nM)
AS (HD1+HD2)
AS (HD1+HD3)
AS (HD1+HD4)
AS (HD1+HD6)
AS (HD1+HD7)
AS (HD1+HD8)
UMM (40nM)
TSA (1uM)

Fig. 16

Human HDAC9 nucleotide sequence (AY032737):

Zhou,X., Richon,V.M., Rifkind,R.A. and Marks,P.A.
Proc. Natl. Acad. Sci. U.S.A. 98 (19), 10572-10577 (2001)
Cloning and characterization of a histone deacetylase, HDAC9

```
   1 atgcacagta tgatcagctc agtggatgtg aagtcagaag ttcctgtggg cctggagccc
  61 atctcacctt tagacctaag gacagacctc aggatgatga tgcccgtggt ggaccctgtt
 121 gtccgtgaga agcaattgca gcaggaatta cttcttatcc agcagcagca acaaatccag
 181 aagcagcttc tgatagcaga gtttcagaaa cagcatgaga acttgacacg gcagcaccag
 241 gctcagcttc aggagcatat caaggaactt ctagccataa aacagcaaca agaactccta
 301 gaaaaggagc agaaactgga gcagcagagg caagaacagg aagtagagag gcatcgcaga
 361 gaacagcagc ttcctcctct cagaggcaaa gatagaggac gagaaagggc agtggcaagt
 421 acagaagtaa agcagaagct tcaagagttc ctactgagta aatcagcaac gaaagacact
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 541 caccacacat cattggatca aagctctcca ccccttagtg gaacatctcc atcctacaag
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 661 gagcccaact tgaaggtgcg gtccaggtta aaacagaaag tggcagagag gagaagcagc
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 901 gagcaaatgg tttcacagca acgcattcta attcatgaag attccatgaa cctgctaagt
 961 ctttatacct ctccttcttt gcccaacatt accttggggc ttcccgcagt gccatcccag
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1681 caggaaatgg aatctgggga gcaggctgct tttatgcaac agcctttcct ggaacccacg
1741 cacacacgtg cgctctctgt gcgccaagct ccgctggctg cggttggcat ggatggatta
1801 gagaaacacc gtctcgtctc caggactcac tcttcccctg ctgcctctgt tttacctcac
1861 ccagcaatgg accgccccct ccagcctggc tctgcaactg gaattgccta tgaccccttg
1921 atgctgaaac accagtgcgt ttgtggcaat tccaccaccc accctgagca tgctggacga
1981 atacagagta tctggtcacg actgcaagaa actgggctgc taaataaatg tgagcgaatt
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2881 atctgtgatg catcagaagc ctgtgtaaat gcccttctag gaaatgagct ggagccactt
2941 gcagaagata ttctccacca aagcccgaat atgaatgctg ttatttcttt acagaagatc
3001 attgaaattc aaagtatgtc tttaaagttc tcttaa
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Human HDAC9 protein sequence (AAK66821)

1 mhsmissvdv ksevpvglep ispldlrtdl rmmmpvvdpv vrekqlqqel lliqqqqqiq
61 kqlliaefqk qhenltrqhq aqlqehikel laikqqqell ekeqkleqqr qeqeverhrr
121 eqqlpplrgk drgreravas tevkqklqef llsksatkdt ptngknhsvs rhpklwytaa
181 hhtsldqssp plsgtspsyk ytlpgaqdak ddfplrktas epnlkvrsrl kqkvaerrss
241 pllrrkdgnv vtsfkkrmfe vtessvssss pgsgpsspnn gptgsvtene tsvlpptpha
301 eqmvsqqril ihedsmnlls lytspslpni tlglpavpsq lnasnslkek qkcetqtlrq
361 gvplpgqygg sipassshph vtlegkppns shqallqhll lkeqmrqqkl lvaggvplhp
421 qsplatkeri spgirgthkl prhrplnrtq saplpqstla qlviqqqhqq flekqkqyqq
481 qihmnkllsk sieqlkqpgs hleeaeeelq gdqamqedra pssgnstrsd ssacvddtlg
541 qvgavkvkee pvdsdedaqi qemesgeqaa fmqqpflept htralsvrqa plaavgmdgl
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661 iqsiwsrlqe tgllnkceri qgrkasleei qlvhsehhsl lygtnpldgq kldprillgd
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781 ghhaeestam gfcffnsvai takylrdqln iskilivdld vhhgngtqqa fyadpsilyi
841 slhrydegnf fpgsgapnev gtglgegyni niawtggldp pmgdveylea frtivkpvak
901 efdpdmvlvs agfdaleght pplggykvta kcfghltkql mtladgrvvl aleggghdlta
961 icdaseacvn allgnelepl aedilhqspn mnavislqki ieiqsmslkf s

Human HDAC10α nucleotide sequence AF407272

Kao,H.Y., Lee,C.H., Komarov,A., Han,C.C. and Evans,R.M.
Isolation and Characterization of Mammalian HDAC10, a Novel Histone Deacetylase
J. Biol. Chem. 277 (1), 187-193 (2002)

1 atggggaccg cgcttgtgta ccatgaggac atgacggcca cccggctgct ctgggacgac
61 cccgagtgcg agatcgagcg tcctgagcgc ctgaccgcag ccctggatcg cctgcggcag
121 cgcggcctgg aacagaggtg tctgcggttg tcagcccgcg aggcctcgga agaggagctg
181 ggcctggtgc acagcccaga gtatgtatcc ctggtcaggg agacccaggt cctaggcaag
241 gaggagctgc aggcgctgtc cggacagttc gacgccatct acttccaccc gagtaccttt
301 cactgcgcgc ggctggccgc aggggctgga ctgcagctgg tggacgctgt gctcactgga
361 gctgtgcaaa atgggcttgc cctggtgagg cctcccgggc accatggcca gagggcggct
421 gccaacgggt tctgcgtgtt caacaacgtg gccatagcag ctgcacatgc caagcagaaa
481 cacgggctac acaggatcct cgtcgtggac tgggatgtgc accatggcca ggggatccag
541 tatctctttg aggatgaccc cagcgtcctt tacttctcct ggcaccgcta tgagcatggg
601 cgcttctggc ctttcctgcg agagtcagat gcagacgcag tggggcgggg acagggcctc
661 ggcttcactg tcaacctgcc ctggaaccag gttgggatgg gaaacgctga ctacgtggct
721 gccttcctgc acctgctgct cccactggcc tttgagtttg accctgagct ggtgctggtc
781 tcggcaggat ttgactcagc catcggggac cctgaggggc aaatgcaggc cacgccagag
841 tgcttcgccc acctcacaca gctgctgcag gtgctggccg gcggccgggt ctgtgccgtg
901 ctggagggcg gctaccacct ggagtcactg gcggagtcag tgtgcatgac agtacagacg
961 ctgctgggtg acccggcccc acccctgtca gggccaatgg cgccatgtca gagtgcccta
1021 gagtccatcc agagtgcccg tgctgcccag gccccgcact ggaagagcct ccagcagcaa
1081 gatgtgaccg ctgtgccgat gagccccagc agccactccc cagaggggag gcctccacct
1141 ctgctgcctg gggtccagt gtgtaaggca gctgcatctg caccgagctc cctcctggac
1201 cagccgtgcc tctgccccgc accctctgtc cgcaccgctg ttgccctgac aacgccggat
1261 atcacattgg ttctgccccc tgacgtcatc caacaggaag cgtcagccct gagggaggag
1321 acagaagcct gggccaggcc acacgagtcc ctggcccggg aggaggccct cactgcactt
1381 gggaagctcc tgtacctctt agatgggatg ctggatgggc aggtgaacag tggtatagca
1441 gccactccag cctctgctgc agcagccacc ctggatgtgg ctgttcggag aggcctgtcc
1501 cacggagccc agaggctgct gtgcgtggcc ctgggacagc tggaccggcc tccagacctc
1561 gcccatgacg ggaggagtct gtggctgaac atcaggggca aggaggcggc tgccctatcc
1621 atgttccatg tctccacgcc actgccagtg atgaccggtg gtttcctgag ctgcatcttg
1681 ggcttggtgc tgcccctggc ctatggcttc cagcctgacc tggtgctggt ggcgctgggg
1741 cctggccatg gcctgcaggg cccccacgct gcactcctga ctgcaatgct tcgggggctg
1801 gcaggggggcc gagtcctggc cctcctggag gagaactcca caccccagct agcagggatc
1861 ctggcccggg tgctgaatgg agaggcacct cctagcctag gcccttcctc tgtggcctcc
1921 ccagaggacg tccaggccct gatgtacctg agagggcagc tggagcctca gtggaagatg

1981 ttgcagtgcc atcctcacct ggtggcttga

human HDAC10α protein sequence (AAK92205):

```
  1 mgtalvyhed mtatrllwdd pecelerper ltaaldrlrq rgleqrclrl sareaseeel
 61 glvhspeyvs lvretqvlgk eelqalsgqf daiyfhpstf hcarlaagag lqlvdavltg
121 avqnglalvr ppghhgqraa angfcvfnnv aiaaahakqk hglhrilvvd wdvhhgqgiq
181 ylfeddpsvl yfswhryehg rfwpflresd adavgrgqgl gftvnlpwnq vgmgnadyva
241 aflhlllpla fefdpelvlv sagfdsaigd pegqmqatpe cfahltqllq vlaggrvcav
301 leggyhlesl aesvcmtvqt llgdpappls gpmapcqsal esiqsaraaq aphwkslqqq
361 dvtavpmsps shspegrppp llpggpvcka aasapsslld qpclcpapsv rtavalttpd
421 itlvlppdvi qqeasalree teawarphes lareealtal gkllylldgm ldgqvnsgia
481 atpasaaaat ldvavrrgls hgaqrllcva lgqldrppdl ahdgrslwln irgkeaaals
541 mfhvstplpv mtggflscil glvlplaygf qpdlvlvalg pghglqgpha alltamlrgl
601 aggrvlalle enstpqlagi larvlngeap pslgpssvas pedvqalmyl rgqlepqwkm
661 lqchphlva
```

Human HDAC10β nucleotide sequence (AF407273)

```
   1 atggggaccg cgcttgtgta ccatgaggac atgacggcca cccggctgct ctgggacgac
  61 cccgagtgcg agatcgagcg tcctgagcgc ctgaccgcag ccctggatcg cctgcggcag
 121 cgcggcctgg aacagaggtg tctgcggttg tcagcccgcg aggcctcgga agaggagctg
 181 ggcctggtgc acagcccaga gtatgtatcc ctggtcaggg agacccaggt cctaggcaag
 241 gaggagctgc aggcgctgtc cggacagttc gacgccatct acttccaccc gagtaccttt
 301 cactgcgcgc ggctggccgc aggggctgga ctgcagctgg tggacgctgt gctcactgga
 361 gctgtgcaaa atgggcttgc cctggtgagg cctcccgggc accatggcca gagggcggct
 421 gccaacgggt tctgcgtgtt caacaacgtg gccatagcag ctgcacatgc caagcagaaa
 481 cacgggctac acaggatcct cgtcgtggac tgggatgtgc accatggcca ggggatccag
 541 tatctctttg aggatgaccc cagcgtcctt tacttctcct ggcaccgcta tgagcatggg
 601 cgcttctggc ctttcctgcg agagtcagat gcagacgcag tggggcgggg acagggcctc
 661 ggcttcactg tcaacctgcc ctggaaccag gttgggatgg gaaacgctga ctacgtggct
 721 gccttcctgc acctgctgct cccactggcc tttgaggggc aaatgcaggc cacgccagag
 781 tgcttcgccc acctcacaca gctgctgcag gtgctggccg gcggccgggt ctgtgccgtg
 841 ctggagggcg gctaccacct ggagtcactg gcggagtcag tgtgcatgac agtacagacg
 901 ctgctgggtg acccggcccc acccctgtca gggccaatgg cgccatgtca gagtgcccta
 961 gagtccatcc agagtgcccg tgctgcccag gccccgcact ggaagagcct ccagcagcaa
1021 gatgtgaccg ctgtgccgat gagccccagc agccactccc cagaggggag gcctccacct
1081 ctgctgcctg gggtccagt gtgtaaggca gctgcatctg caccgagctc cctcctggac
1141 cagccgtgcc tctgccccgc accctctgtc cgcaccgctg ttgccctgac aacgccggat
1201 atcacattgg ttctgccccc tgacgtcatc caacaggaag cgtcagccct gagggaggag
1261 acagaagcct gggccaggcc acacgagtcc ctggcccggg aggaggccct cactgcactt
1321 gggaagctcc tgtacctctt agatgggatg ctggatgggc aggtgaacag tggtatagca
1381 gccactccag cctctgctgc agcagccacc ctggatgtgg ctgttcggag aggcctgtcc
1441 cacggagccc agaggctgct gtgcgtggcc ctgggacagc tggaccggcc tccagacctc
1501 gcccatgacg ggaggagtct gtggctgaac atcaggggca aggaggcggc tgccctatcc
1561 atgttccatg tctccacgcc actgccagtg atgaccggtg gtttcctgag ctgcatcttg
1621 ggcttggtgc tgcccctggc ctatggcttc cagcctgacc tggtgctggt ggcgctgggg
1681 cctggccatg gcctgcaggg cccccacgct gcactcctga ctgcaatgct tcgggggctg
1741 gcaggggggcc gagtcctggc cctcctggag gagaactcca caccccagct agcagggatc
1801 ctggcccggg tgctgaatgg agaggcacct cctagcctag gcccttcctc tgtggcctcc
1861 ccagaggacg tccaggccct gatgtacctg agagggcagc tggagcctca gtggaagatg
1921 ttgcagtgcc atcctcacct ggtggcttga
```

Human HDAC10β protein sequence (AAK92206):

```
  1 mgtalvyhed mtatrllwdd pecelerper ltaaldrlrq rgleqrclrl sareaseeel
 61 glvhspeyvs lvretqvlgk eelqalsgqf daiyfhpstf hcarlaagag lqlvdavltg
121 avqnglalvr ppghhgqraa angfcvfnnv aiaaahakqk hglhrilvvd wdvhhgqgiq
181 ylfeddpsvl yfswhryehg rfwpflresd adavgrgqgl gftvnlpwnq vgmgnadyva
241 aflhlllpla fegqmqatpe cfahltqllq vlaggrvcav leggyhlesl aesvcmtvqt
301 llgdpappls gpmapcqsal esiqsaraaq aphwkslqqq dvtavpmsps shspegrppp
```

```
361 llpggpvcka aasapsslld qpclcpapsv rtavalttpd itlvlppdvi qqeasalree
421 teawarphes lareealtal gkllylldgm ldgqvnsgia atpasaaaat ldvavrrgls
481 hgaqrllcva lgqldrppdl ahdgrslwln irgkeaaals mfhvstplpv mtggflscil
541 glvlplaygf qpdlvlvalg pghglqgpha alltamlrgl aggrvlalle enstpqlagi
601 larvlngeap pslgpssvas pedvqalmyl rgqlepqwkm lqchphlva
```

Human HDAC11: sequence disclosed in manuscript in press, see attached.